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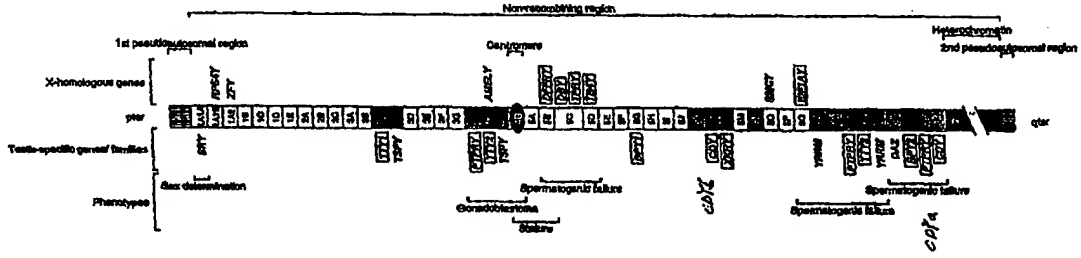

(57) Abstract

Genes of the non–recombining region of the human Y chromosome, which fall into two classes: X–homologous DNA which is expressed in many organs and has functional X homologs and testis–specific DNA.

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GENES IN THE NON-RECOMBINING REGION OF THE Y CHROMOSOME

GOVERNMENT SUPPORT

The invention described herein was made in whole or in part with government support under Grant Number HG00257 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/041,877, filed April 11, 1997, entitled "Genes in the Non-Recombining Region of the Y Chromosome" by Bruce T. Lahn and David C. Page. The entire teachings of the above referenced application is expressly incorporated herein by reference.

BACKGROUND OF THE INVENTION

The human Y chromosome is distinguished from all other nuclear chromosomes by four characteristics: the absence of recombination, its presence in males only, its common ancestry and persistent meiotic relationship with the X chromosome, and the tendency of its genes to degenerate during evolution (J. J. Bull, *Evolution of Sex Determining Mechanisms* (Benjamin Cummings, Menlo Park, CA, 1983); J. A. Graves, *Annu. Rev. Genet. 30:*233 (1996); B. Charlesworth, *Curr. Biol. 6:*149 (1996); W. R. Rice, *BioScience, 46,* 331

(1996)). To be precise, these distinctive characteristics apply only to the non-recombining portion or region of the Y chromosome (NRY), which comprises 95% of the human Y chromosome. The remaining 5% of the chromosome is composed of two pseudoautosomal regions that maintain sequence identity with the X chromosome by meiotic recombination (H. J. Cooke *et al.*, *Nature 317:*687 (1985); M. C. Simmler *et al.*, *Nature 317:*692 (1985); D. Freije *et al.*, *Science 258:*1784 (1992); G. A. Rappold, *Hum. Genet. 92:*315 (1993)). Given the NRY's peculiar characteristics, one might expect its gene content to be idiosyncratic. Since discovery of the Y chromosome in 1923, its gene content has been the subject of speculation. By the middle of this century, while studies of human pedigrees had identified many traits exhibiting autosomal or X-linked inheritance, no convincing cases of Y-linked inheritance could be found (T. S. Painter, *J. Exp. Zool.* (1923); C. Stern, *Am. J. Hum. Genet. 9:*147 (1957)). As a result, consensus began to emerge that the Y chromosome carried few, if any, genes. In 1959, reports of XO females and XXY males established the existence of a sex-determining gene on the human Y chromosome (P. A. Jacobs *et al. Nature 183:*302 (1959); C. E. Ford *et al.*, *Lancet, i:*711 (1959)), but this was perceived as a special case on a generally desolate chromosome. Opinions began to change only during the past decade, when eight NRY transcription units (or families of closely related transcription units) were identified, most during regionally focused, positional cloning experiments (D. C. Page *et al.*, *Cell 51:*1091 (1987); A. H. Sinclair *et al.*, *Nature 346:*240-244 (1990); J. Arnemann *et al.*, *Genomics 11:* 108 (1991); E. C. Salido *et al.*, *Am. J. Hum. Genet. 50:*303 (1992); E. M. Fisher *et al.*, *Cell 63:*1205 (1990); K. Ma *et al.*, *Cell 75:*1287 (1993); A. I. Agulnik *et al.*, *Hum. Mol. Genet. 3:*879 (1994); R. Reijo *et al.*, *Nat.*

Genet. 10:383 (1995)). It was not known if there were more genes in the NRY.

SUMMARY OF THE INVENTION

A systematic search of the non-recombining region of the human Y chromosome (NRY) has identified 12 novel genes or gene families. All 12 novel genes, and six of eight NRY genes or families previously isolated by less systematic means, fall into two classes. The first class of genes exists in one copy and is expressed in many organs; they have functional X homologs that escape X inactivation, as predicted for genes involved in Turner (XO) syndrome. The second class consists of Y-chromosomal gene families expressed specifically in testes, and may account for infertility among men with Y deletions.

The genes described herein, portions of the genes and DNA which hybridizes to genes or gene portions described are useful in diagnostic methods, such as a method to identify individuals in whom all or a portion of a gene or genes of the NRY is missing or altered. For example, Y chromosomal DNA from males with a known condition, such as infertility or reduced sperm count, can be assessed, using the gene(s) described herein, or characteristic portions thereof, to determine whether their DNA lacks some or all of the gene(s) described herein or contains an altered gene(s) (e.g., a gene in which there is a deletion, substitution, addition or mutation, compared to the sequences presented herein). Y chromosomal DNA (e.g., from a male with reduced sperm count or viability) can be assessed, using DNA described herein or DNA which hybridizes to DNA described herein, to determine whether the condition is associated with or caused by the occurrence of the gene or the gene alteration. For example, the presence or absence of all or a portion of a gene or genes shown to be necessary for fertility or

adequate sperm count can be assessed, using DNA which hybridizes to the gene or genes of interest to determine the basis for their infertility or reduced sperm count. In one embodiment, the occurrence of one or more Y-specific genes or a characteristic portion of one or more Y-specific genes is assessed in Y chromosomal DNA. In another embodiment, deletion or alteration of one of the testis-specific (Y-specific) genes described is assessed, such as by a hybridization method in which DNA which hybridizes to one of the Y-specific genes described herein or a characteristic portion thereof is used to assess a DNA sample obtained from a male who has a reduced sperm count. Lack of hybridization of the Y-specific DNA used to DNA in the sample indicates that the gene is not present in sample DNA or is present in an altered form which does not hybridize to Y-specific DNA of the present invention. In another embodiment, an X-homologous gene or genes present on the NRY can be used to determine whether the gene is present in an individual or if it occurs in an altered form in the individual. Using known methods, such as hybridization methods, X or Y chromosomal DNA from an individual can be assessed for the presence or absence of one or more of the X-homologous genes or a characteristic portion of one or more X-homologous genes. X or Y chromosomal DNA can also be assessed for the presence or absence of an altered form of one or more of the X-homologous genes described. In the present methods, DNA can be analyzed for the occurrence of Y-specific DNA, X-homologous genes or both. For example, a "battery" or group of DNA probes (sequences) can be used to analyze sample DNA; the probes can include Y-specific DNA probes (e.g., DNA which hybridizes to a Y-specific gene), X-homologous gene probes (e.g., DNA which hybridizes to an X-homologous gene) or both types of probes. DNA described herein is also useful as primers in an amplification

method, such as PCR, useful for identifying and amplifying Y-specific DNA or X-homologous genes in a sample (e.g., Y chromosomal DNA). Further, proteins or peptides encoded by the DNA described herein, such as proteins or peptides encoded by an X-homologous gene or proteins or peptides encoded by testis-specific DNA (a testis-specific gene), can be assessed in samples. This can be carried out, for example, using antibodies which recognize proteins or peptides of the present invention (proteins or peptides encoded by DNA described herein).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a gene map of the non-recombining region of the Y chromosome.

Figure 2 shows the amino acid sequence alignments of the chromodomain (SEQ ID NO.: 1-6) and putative catalytic domain (SEQ ID NO.: 7-12) of human CDY genes with their respective homologs. Amino acid identities are indicated by black shading and for each protein, the first and last amino acid residues are numbered (with respect to the initiator methionine) and the total length of the protein is indicated. Chromodomain: SEQ ID NO.: 1, CDY (human); SEQ ID NO.: 2, HP1 (Drosophila); SEQ ID NO.: 3, Polycomb (Drosophila); SEQ ID NO.: 4, CHD1 (Drosophila); SEQ ID NO.: 5, Su(var) 3-9 (Drosophila; SEQ ID NO.: 6, PDD1 (Tetrahymena); SEQ ID NO.: 7; Covalent modification domain: SEQ ID NO.: 8, CDY (human); SEQ ID NO.: 9, Enoyl-CoA Hydratase (Human); SEQ ID NO.: 10, 4-CBA-CoA dehalogenase (Arthrobacter); SEQ ID NO.: 11, Crotonase (C. acetobutylicum); SEQ ID NO.: 12, Naphthoate synthase (E. coli).

Figures 3A and 3B are the nucleic acid sequence of DBX (long and short transcripts, SEQ ID NO: 13 and SEQ ID NO: 14, respectively) and the encoded amino acid sequences (SEQ ID NO: 15 and SEQ ID NO.: 16, respectively), DBY (SEQ ID

NO: 17) and the encoded amino acid sequence (SEQ ID NO: 18). Dots in the DBX DNA and protein sequences indicate that the nucleic acids or amino acid residues are the same as those represented for DBY; dashes indicate a missing nucleic acid or amino acid residue.

Figures 4A and 4B present the nucleic acid sequences for three forms of TPRY (short, medium and long, SEQ ID NO: 19, SEQ ID NO: 20 and SEQ ID NO: 21, respectively) and the encoded amino acid sequences for the short, medium and long forms (SEQ ID NO: 22, SEQ ID NO.: 23 and SEQ ID NO: 24, respectively).

Figure 5 presents the nucleic acid sequences of TB4X (SEQ ID NO: 25) and TB4Y (SEQ ID NO: 26) and the encoded amino acid sequences (SEQ ID NO: 27 and SEQ ID NO: 28, respectively). Dots in the TB4X DNA and protein sequences indicate that the nucleic acids or amino acid residues are the same as those represented for TB4Y.

Figure 6 represents the nucleic acid sequences of EIF1AX (SEQ ID NO: 29) and EIF1AY (SEQ ID NO: 30) and the encoded amino acid sequences (SEQ ID NO: 31 and SEQ ID NO: 32, respectively).

Figures 7A - 7D represent the nucleic acid sequences of DFFRX (SEQ ID NO: 33) and DFFRY (SEQ ID NO: 34) and the encoded amino acid sequences (SEQ ID NO: 35 and SEQ ID NO: 36, respectively).

Figure 8 represents the nucleic acid sequences of CDYa (SEQ ID NO: 37) and CDYb (SEQ ID NO: 38) and the encoded amino acid sequences (SEQ ID NO: 39 and SEQ ID NO: 40, respectively).

Figure 9 represents the nucleic acid sequences of BPY1 (SEQ ID NO: 41) and the encoded amino acid sequence (SEQ ID NO: 42).

Figure 10 represents the nucleic acid sequence of BPY2 (SEQ ID NO: 43) and the encoded amino acid sequence (SEQ ID NO: 44).

Figure 11 represents the nucleic acid sequences of XKRY (SEQ ID NO: 45) and the encoded amino acid sequence (SEQ ID NO: 46).

Figure 12 represents the nucleic acid sequences of PTPRY (SEQ ID NO: 47) and the encoded amino acid sequence (SEQ ID NO: 48).

Figure 13 is the nucleic acid sequence of TTY1 (SEQ ID NO: 49).

Figure 14 is the nucleic acid sequence of TTY2 (SEQ ID NO: 50).

Figure 15 shows the nucleic acid sequence of the human CDY Like (CDYL) gene, which is the human autosomal homolog of CDY, located on chromosome 6p and expressed ubiquitously.

Figure 16 shows the nucleic acid sequence of the mouse Cdyl (CDY like) gene, which is the mouse ortholog of human CDYL, located on chromosome 13 and expressed predominantly in the testis. A longer transcript of the gene is ubiquitously expressed.

Figures 17A - 17C show the nucleic acid sequences of human Variably Charged Protein family members VCP2r, VCP8r and VCP10r, which are expressed in the testis and highly polymorphic.

Figure 17A is the nucleic acid sequence of VCP2r.

Figure 17B is the nucleic acid sequence of VCP8r.

Figure 17C is the nucleic acid sequence of VCP10r.

DETAILED DESCRIPTION OF THE INVENTION

Y chromosome genes, classed as genes having X homologues and testis-specific (Y-specific) genes, are the subject of the invention described herein, as are DNA which hybridize to (are complementary to) all or characteristic portions of the Y chromosome genes, the encoded products (e.g., proteins, peptides, glycoproteins), antibodies and methods of diagnosis or treatment in which the genes,

complementary DNA, encoded proteins or antibodies are used. As described herein, fragments that hybridized to Y chromosomal DNA were selected and then their nucleotide sequences determined. It was expected that these sequence fragments would represent a redundant sampling of a much smaller set of genes. Computer analysis revealed that 577 fragments corresponded to known Y genes, including seven of eight NRY genes and all eight pseudoautosomal genes previously reported. These findings suggested that the 2539 sequence fragments represented the great majority of all Y-chromosomal genes. After further analysis, both to eliminate human repetitive sequences and to assemble overlapping fragments into contigs, 912 novel and non-overlapping sequences were hybridized to Southern blots of human genomic DNAs. 308 sequences that detected at least one prominent male-specific fragment were judged likely to derive from the NRY, and for each work was carried out to isolate cDNA clones from a human testis library, as described in Example 1. Nucleotide sequencing of cDNA clones, and rescreening of libraries as necessary, yielded full-length cDNA sequences for ten novel NRY genes or families, and partial cDNA sequences for two additional ones (Table and Figures 1 - 14).

TABLE: 12 Novel Genes or Families in the NRY

Gene Symbol	Gene Name	Tissue Expression	Multi-copy on Y	X homolog	Escape x Inactivation
DBY	Dead Box Y	ubiquitous		DBX	yes
TB4Y	Thymosin β4, Y isoform	ubiquitous		TB4X	yes
EIF1AY	Translation Initiation Factor 1A, Y isoform	ubiquitous		EIF1AX	yes
TPRY	TPR motif Y	ubiquitous		TPRX	yes
DFFRY	Drosophila Fat Facets Related Y	ubiquitous		DFFRX	yes
CDY	Chromodomain Y	testis	yes		
BPY1	Basic Protein Y 1	testis	yes		
BPY2	Basic Protein Y 2	testis	yes		
XKRY	XK Related Y	testis	yes		
PTPRY	Protein-Tyrosine Phosphatase Related Y	testis	yes		
TTY1	Testis Transcript Y 1	testis	yes		
TTY2	Testis Transcript Y 2	testis	yes		

All 12 novel genes were localized on the Y chromosome, as described in Example 2. Figure 1 is a gene map of NRY. As shown, the Y chromosome consists of a large non-recombining region (NRY; euchromatin plus heterochromatin) flanked by pseudoautosomal regions (pter, short arm telomere; qter, long arm telomere). The NRY is divided into 43 ordered intervals (1A1A through 7) which are defined by naturally occurring deletions (D. Vollrath, *et al.*, *Science 258:*52 (1992)). Listed immediately above the Y chromosome in Figure 1 are nine NRY genes with functional X homologs; novel genes are boxed. Indicated immediately below the Y chromosome are 11 testis-specific genes or families, some with multiple locations. It is likely that some testis-specific families have members in additional deletion intervals; the locations indicated are representative, but are not necessarily exhaustive. At the bottom of Figure 1 are shown NRY regions implicated, by deletion mapping, in sex determination, germ cell tumorigenesis (gonadoblastoma), stature, and spermatogenic failure (K. Ma *et al.*, *Cell 75:*1287 (1993); R. Reijo *et al.*, *Nat. Genet. 10:*383 (1995); P. H. Vogt *et al.*, *Hum. Mol. Genet. 5:*933 (1996); J. L. Pryor *et al.*, *New England J. Med. 336:*534 (1997); K. Tsuchiya *et al.*, *Am. J. Hum. Genet. 57:*1400 (1995); P. Salo *et al.*, *Hum. Genet. 95:*283 (1995)). Euchromatic regions that are made up, at least partially, of Y-specific repeats are drawn in grey. *AMELY*, which appears to fall within such a repeat-containing region, is actually located in a sub-region of 4A that is not repetitive.

Expression of the 12 novel genes was assessed in diverse human tissues, by Northern blotting. Autoradiograms were produced by hybridizing ^{32}P-labeled cDNA probes to Northern blots of poly(A)$^+$ RNAs (2 μg/lane) from human tissues (Clontech, Palo Alto, CA). Probes employed were cDNA clones, full-length (most genes) or

partial (*DBY*, nucleotides 1476-2319 of GenBank AF000985; *TPRY*, nucleotides 861-1768 of GenBank AF000996; *DFFRY*, nucleotides 8604-9878 of GenBank AF000986). Blots were hybridized at 65°C in Church's buffer (0.5 M Na_iPO_4 at pH7.5, with 7% SDS), and washed at 65°C in 1X SSC and 0.1% SDS. *DBY*, *TB4Y*, *EIF1AY* and *DFFRY* probes cross-hybridize to transcripts derived from their X homologs. For all five X-homologous genes (*DBY*, *TPRY*, *TB4Y*, *EIF1AY* and *DFFRY*), expression was tested and confirmed in three male tissues (brain, prostate and testis) by RT-PCR using Y-specific primers.

The novel genes encode an assortment of proteins and are dispersed throughout the euchromatic portions of the NRY. Nonetheless, all 12 genes fall into two discrete classes: 1) X-homologous genes and 2) testis-specific, Y-specific gene families (Table).

The X-homologous genes share the following characteristics: each has a homolog on the X chromosome encoding an extremely similar but nonidentical protein isoform, each is expressed in a wide range of human tissues (is not testis-specific), and each appears to exist in a single copy on the NRY. There are five novel representatives of this X-homologous class:

1. *DBY* encodes a novel "DEAD box" protein, perhaps an RNA helicase involved in translation initiation (P. Linder, et al., *Nature, 337*, 121 (1989); R.-Y. Chuang, P. L. Weaver, Z. Liu, T.-H. Chang, *Science, 275*, 1468 (1997)). The DBY protein is 91% identical to DBX, encoded by a homologous gene on the human X chromosome.
2. *TPRY* encodes a novel protein containing 10 tandem "TPR" motifs, a protein-protein interaction domain found in the products of the yeast SSN6/CYC8, CDC16, and CDC23 genes, among others (R. S. Sikorski, M. S. Boguski, M. Goebl, P. Hieter, *Cell, 60*, 307 (1990); D. Tzamarias, K. Struhl, *Genes Dev, 9*, 821 (1995)). Differential splicing may

generate TPRY isoforms that differ at their carboxy termini. The amino terminal portion of the TPRY protein is 83% identical to TPRX, encoded by an homologous gene on the X chromosome.

3. *TB4Y* encodes a 44 amino acid protein that differs at only three residues from thymosin $ß_4$, which functions in actin sequestration (H. Gondo, et al., *J. Immunol. 139:*3840 (1987); D. Safer, M. Elzinga, V. T. Nachmias, *J Biol Chem, 266,* 4029 (1991)), and we found is located on the X. It is proposed that the X-linked gene encoding thymosin $ß_4$ be called *TB4X.*

4. *EIF1AY* encodes a Y-linked isoform of translation initiation factor 1A (eIF-1A) (T. E. Dever, et al., *J Biol Chem, 269,* 3212 (1994); J. W. Hershey, *Annu. Rev. Biochem. 60,* 717 (1991)), which we discovered is located on the X. It is proposed that the X-linked gene encoding eIF-1A be called *EIF1AX.* The amino acid sequences of the X and Y-encoded proteins are 97% identical.

5. *DFFRY* encodes a Y-linked isoform of DFFRX, a recently described X-linked protein. A Y-linked homolog was detected previously, but had been thought to be a pseudogene. The human DFFRX and DFFRY proteins, which are 91% identical, are homologous to the Drosophila *fat-facets* gene product, a deubiquinating enzyme required for eye development and oogenesis (M. H. Jones, et al., *Hum Mol Genet* 5, 1695 (1996); J. A. Fischer-Vize, G. M. Rubin, R. Lehmann, *Development, 116,* 985 (1992); Y. Huang, R. T. Baker, J. A. Fischer-Vize, *Science, 270,* 1828 (1995)).

The second group of novel NRY genes, the testis-specific, Y-specific gene families, share a very different set of characteristics: each appears to be expressed specifically in testes and each appears to exist in multiple copies on the NRY, as judged by I) the number and intensity of hybridizing fragments on genomic Southern blots or ii) multiple map locations on the Y. We report

five novel testis-specific, Y-specific gene families with full-length cDNA sequences:

1. The *CDY* family encodes proteins with an amino-terminal "chromodomain," a chromatin binding motif (T. C. James, S. C. Elgin, *Mol Cell Biol, 6,* 3862 (1986); B. Tschiersch, et al., *EMBO J, 13,* 3822 (1994); R. Paro, D. S. Hogness, *Proc Natl Acad Sci U S A, 88,* 263 (1991); D. G. Stokes, K. D. Tartof, R. P. Perry, *Proc Natl Acad Sci U S A, 93,* 7137 (1996); M. T. Madireddi, et al., *Cell, 87,* 75 (1996)) (Figure 3). The carboxy-terminal half shows striking amino acid similarity, over a region of more than 200 residues, to nearly the full length of several enzymes, both prokaryotic and eukaryotic (M. Kanazawa, et al., *Enzyme Protein, 47,* 9 (1993); A. Schmitz, K. H. Gartemann, J. Fiedler, E. Grund, R. Eichenlaub, *Appl. Environ. Microbiol. 258,* 4068 (1992); Z. L. Boynton, G. N. Bennet, F. B. Rudolph, *J Bacteriol, 178,* 3015 (1996); V. Sharma, K. Suvarna, R. Meganathan, M. E. Hudspeth, *J Bacteriol, 174,* 5057 (1992); P. M. Palosaari, et al., *J Biol Chem, 266,* 10750 (1991)). The reactions catalyzed by these homologs are diverse, but in each case the substrate contains cofactor A (CoA) attached to a carbonyl group, and an alkoxide intermediate is formed. The unprecedented combination of a chromodomain and a putative CoA-substrate enzyme in a single polypeptide suggests that, in vivo, CDY proteins may catalyze covalent modification of DNA or chromosomal proteins, perhaps during spermatogenesis.

2. The *BPY1* genes encode a basic protein, 125 residues long, with little sequence similarity to known proteins. The encoded protein is rich in serine, lysine, arginine, and proline and has a pI of 9.4. Southern blotting studies revealed homologous sequences on the human X chromosome, but screening of cDNA libraries has failed to yield X-derived clones.

3. The *BPY2* genes encode a second basic protein, 106 residues in length, without obvious sequence similarity to BPY1 or other known proteins. The pI of BPY2 is 10.0.

4. The *XKRY* genes encode a protein with sequence similarity to XK, a putative membrane transport protein defective in McLeod syndrome (M. Ho, et al., *Cell*, 77, 869 (1994)).

5. The *PTPRY* genes encode a protein with weak homology to a putative protein-tyrosine phosphatase (PTPase) in the mouse (W. Hendriks, et al., *J Cell Biochem*, 59, 418 (1995)). Two additional families of testis-specific transcription units, referred to as *TTY1* and *TTY2*, have been identified. The sequences represented in Figures 14 and 15 are being assessed for open reading frames.

It appears that conventional single-copy genes, commonplace elsewhere in the genome, are quite uncommon in the NRY. Indeed, the two classes of NRY genes suggested by the systematic search described herein accommodate not only the 12 genes reported here, but also six of eight previously identified NRY genes. *SRY*, a Y-specific gene that triggers the male pathway of sexual differentiation, is expressed in testes, and exists in only one copy in the NRY. *AMELY*, which has an X-linked homolog *AMELX*, is expressed only in the developing tooth bud. The X inactivation status of *AMELX* is unknown.

Also described herein are five additional genes and their sequences (Figures 15, 16, 17A - 17C): human CDY Like (CDYL), which is the human homolog of CDY; it is on chromosome 6p and expressed ubiquitously; mouse Cdyl (CDY like), which is the mouse ortholog of human CDYL; it is on chromosome 13 and expressed predominantly in testis and also has a longer transcript that is expressed ubiquitously; and human VCP (Variably Charged Protein) family, which is a family of genes on the X chromosome that are homologous to BPYI, expressed in the testis and highly

polymorphic. Human CDY, human CDYL and mouse Cdyl have been shown to be histone acetyltransferases by *in vitro* assays. Human CDY is a candidate for the Azoospermia Factor (AZF) because it is within the AZFc region that is commonly deleted in infertile men. Chemicals that block the enzymatic activity of any of these genes are candidate male contraceptives.

Inhibitors of the enzymatic activity of these genes, such as the human CDY gene, can be identified through an *in vitro* assay. For example, the protein encoded by one of the genes (e.g., CDY-encoded protein) can be produced, such as by recombinant means (e.g., in bacterial cells containing a vector or plasmid which includes the gene to be expressed), and obtained. The effect of a candidate inibitor (drug) on the enzymatic activity of the protein can be assessed by combining the candidate inhibitor with the protein, a substrate of its enzymatic activity (e.g., histones) acetyl CoA (e.g., radiolabelled acetyl CoA) and other assay components (e.g., an appropriate physiological solution or buffer), to produce a combination. The combination is maintained under conditions under which the enzymatic activity of the protein is maintained and appropriate for the protein to act upon/interact with its substrate (e.g., for the CDY gene to retain its histone acetyltransferase activity). As a result, the substrate is acted upon by the protein if the candidate inhibitor does not inhibit the protein and the protein acts upon the substrate. If the substrate is not acted upon by the protein, this is an indication that the candidate inhibitor is an inhibitor of the protein. For example, if a histone acetyltransferase, such as CDY-encoded protein is inhibited by a candidate inhibitor, its histone acetyltransferase activity will be blocked. If radiolabelled acetyl CoA is used, transfer of the radiolabelled acetyl group to the enzyme substrate (histones) is inhibited (will not occur or

will occur to a lesser extent than occurs in the absence of the candidate inhibitor). Whether transfer occurs can be assessed by determining the location of radiolabelled acetyl groups from acetyl CoA. If the histone substrates are not radiolabelled or are radiolabelled to a lesser extent in the presence of a candidate inhibitor (than in its absence), the candidate inhibitor is an inhibitor of the protein. Inhibitors identified in this way can be further assessed in additional *in vitro* assays or in *in vivo* assays (e.g., in an appropriate animal model).

To interpret the observation that these X-homologous and multi-copy, testis-specific groups account for 18 of 20 known NRY genes or families, we postulate that the NRY's evolution was dominated by two strategies. The first strategy favors conservation of certain existing genes and the second favors the acquisition of a class of novel genes: 1) The X-homologous genes probably reflect the common ancestry of the X and Y chromosomes, and selective pressures to maintain comparable expression of genes in males and females. 2) The abundance of testis-specific families may have resulted from the NRY's selectively retaining and amplifying genes that enhance male reproductive fitness.

1) Dosage compensation and X-Y homology. Experts agree that the mammalian X and Y chromosomes evolved from autosomes, with nearly all ancestral gene functions deteriorating on the non-recombining portion of the emerging Y chromosome while being maintained on the nascent X chromosome (J. J. Bull, *Evolution of Sex Determining Mechanisms* (Benjamin Cummings, Menlo Park, CA, 1983); J. A. Graves, *Annu. Rev. Genet. 30:*233 (1996); B. Charlesworth, *Curr. Biol. 6:*149 (1996); W. R. Rice, *BioScience 46:*331 (1996)). Functional degeneration of the NRY would result in females having two, but males only one, copy of many genes, creating the need for a mechanism to equalize

X-linked gene expression in the sexes. In mammals, a predominant solution to this problem is provided by X inactivation, the transcriptional silencing of one X chromosome in females.

However, the findings on X-homologous NRY genes described herein, combined with previous studies, illustrate the importance in human evolution of an alternative solution: preservation of homologous genes on both the NRY and the X chromosome, with both male and female cells expressing two copies of such genes. A critical prediction of this model is that, in female cells, the X homologs should escape X inactivation. This is the case for all widely expressed X-linked genes with known NRY homologs, including the X homologs of five novel NRY genes reported here (E. M. Fisher, *et al.*, *Cell 63*:1205 (1990); A. I. Agulnik*et al.*, *Hum. Mol. Genet. 3*:879 (1994); M. H. Jones *et al.*, *Hum. Mol. Genet. 5*:1695 (1996); J. A. Fischer-Vize *et al.*, *Development 116*:985 (1992); Y. Huang *et al.*, *Science 270*:1828 (1995); A. Schneider-Gädicke *et al.*, *Cell 57*:1247 (1989)). A second prediction of this model is that the human X and Y encoded proteins should be functionally interchangeable even though the nucleotide sequences of their corresponding genes are considerably diverged. Indeed, each of the eight known X-NRY gene pairs encode closely related isoforms, with 83 to 97% amino acid identity throughout their lengths; functional interchangeability has been demonstrated in the one case tested to date (M. Watanabe *et al.*, *Nat. Genet. 4*:268 (1993)).

Turner syndrome is classically associated with an XO sex chromosome constitution. In 1965, Ferguson-Smith postulated that the Turner phenotype might be due to inadequate expression of X-Y common genes that escape X inactivation (M. A. Ferguson-Smith, *J. Med. Genet. 2*:142 (1965)). These "Turner genes" have yet to be identified

with certainty. However, there now exists a substantial collection of X-homologous NRY genes (Figure 1) which can be assessed for genes which contribute to or are responsible for the Turner phenotype. The potential role of *RPS4Y* and *RPS4X* in Turner syndrome is controversial (E. M. Fisher *et al.*, *Cell 63:*1205 (1990); W. Just *et al.*, *Hum. Genet. 89:*240 (1992)). At least one Turner gene maps to the Xp-Yp pseudoautosomal region (T. Ogata *et al.*, *J. Med. Genet. 30:*918 (1993)). Seven of the eight known X-NRY gene pairs appear to be ubiquitously expressed, and at least three encode housekeeping proteins: an essential ribosomal protein (RPS4), an essential translation initiation factor (eIF-1A), and a modulator of actin polymerization (thymosin ß4). Perhaps some features of the XO phenotype (e.g., poor fetal viability) reflect inadequate expression of such housekeeping functions.

2) Male fitness and Y-specific, testis-specific genes. As first appreciated by R.A. Fisher, animal genomes may contain genes or alleles that enhance male reproductive fitness but are inconsequential or detrimental with respect to female fitness (R. A. Fisher, *Biol. Rev. 6:*345 (1931)). As Fisher recognized, selective pressures would tend to favor the accumulation of such genes in male-specific regions of genomes. Of course, male reproductive fitness depends critically on sperm production, the central task of the adult testis. Since the NRY is the only male-specific portion of the mammalian genome, it should have a unique tendency to accumulate male-benefit genes during evolution.

These principles are illustrated by several gene families on the human NRY. *De novo* deletions of the *DAZ* gene cluster on the human Y chromosome are associated with severe spermatogenic defects (R. Reijo *et al.*, *Nat. Genet. 10:*383 (1995)), and in *Drosophila* the *DAZ* homolog *boule* is required for spermatogenesis (C. G. Eberhart *et al.*, *Nature 381:*783 (1996)). The *DAZ* gene cluster on the human Y

chromosome arose, during primate evolution, by transposition and amplification of an autosomal gene. Likewise, two other testis-specific NRY gene families —*YRRM* and *TSPY* — may also be the result of the Y chromosome's having acquired and amplified autosomal genes (R. Saxena *et al.*, *Nat. Genet. 14*:292 (1996); M. L. Delbridge *et al.*, *Nat. Genet. 15*:131 (1997)). It is possible that the selective advantage conferred by the NRY's retaining and amplifying male fertility factors (from throughout the genome) accounts for the multitude of testis-specific gene families there. This may have been the preeminent force in shaping the NRY's gene repertoire, as it appears that the great majority of NRY transcription units are members of such testis-specific families. In the NRY, each of the testis-specific gene families has multiple members, 20 to 40 copies in the case of *TSPY* (E. Manz *et al.*, *Genomics 17:* 726 (1993)), and perhaps as many as 20 copies in the case of *YRRM* (K. Ma *et al.*, *Cell 75*:1287 (1993)). All together, the various Y-specific gene families may include as many as several hundred genes or copies. Though it is not known how many of these are functional, it seems likely that Y-specific, testis-specific gene families comprise the great majority of NRY transcription units.

Recent genetic studies underscore the importance of the human Y chromosome in fertility. Many men with spermatogenic failure, but who are otherwise healthy, have deletions of portions of the NRY (K. Ma *et al.*, *Cell 75:* 1287 (1993); R. Reijo *et al.*, *Nat. Genet. 10*:383 (1995); P. H. Vogt *et al.*, *Hum. Mol. Genet. 5*:933 (1996); J. L. Pryor *et al.*, *New England J. Med. 336*:534 (1997)). These findings suggested the existence of NRY genes that play critical roles in male germ cell development but are not required elsewhere in the body. Previous deletion mapping studies have implicated four regions of the NRY in either

spermatogenic failure or germ cell tumorigenesis, and in each of these four regions we now report novel candidate genes expressed specifically, or most abundantly, in testes (Figure 1). As shown in Figure 1, the region implicated in gonadoblastoma, stature and spermatogenic failure all contain novel candidate genes. Two of the three regions implicated in spermatogenic failure each contain one or more novel testis-specific genes. The third region implicated in spermatogenic failure (intervals 5B-5D) contains two X-homologous genes, *DBY* and *EIF1AY*, with abundant, testis-specific transcripts in addition to higher-molecular-weight, ubiquitous transcripts.

While X-homologous and testis-specific genes are somewhat intermingled within the NRY, clustering is evident (Figure 1). The geographic distribution of the two classes correlates quite well with previously identified sequence domains within the euchromatic NRY (D. Vollrath *et al.*, *Science 258:*52 (1992); S. Foote *et al.*, *Science 258:*60 (1992)). Ten of the 11 known testis-specific families map to previously identified regions of Y-specific repetitive sequences. The only exception is *BPY1*, which cross-hybridizes to the X chromosome and maps to a previously recognized region of X homology. Indeed, one or more testis-specific gene families are found in nearly all known regions of euchromatic Y repeats (Figure 1). Ironically, it had been widely assumed that these regions consisted of "junk" DNA, partly on theoretical grounds (B. Charlesworth, *Science 251:*1030 (1991); E. Seboun *et al.*, *Cold Spring Harb. Symp. Quant. Biol. 1:*237 (1986)). To the contrary, the results presented here argue that these Y-specific repetitive regions contain the great majority of the NRY's transcription units (The only exception is *BPY1*, which cross-hybridizes to the X chromosome and maps to a previously recognized region of X homology). These regions may be the result of rampant gene amplification during

mammalian evolution. By contrast, none of the eight X-homologous genes map to the Y-repeat regions; all eight map to regions previously identified as consisting largely of single-copy (or in some cases X-homologous) sequences. It is possible that, early in mammalian evolution, these regions of the NRY shared extensive sequence identity with the nascent X chromosome. The stage is now set for systematic evolutionary, biochemical and cell biological studies of the NRY, an idiosyncratic segment of the human genome.

The present invention relates to isolated DNA and genes, present on (which occur on) the Y chromosome, whose sequences are provided herein, as well as characteristic portions of the DNA. It relates to additional nucleic acid/nucleotide sequences which are not identical to the sequences presented herein but include substitutions or differences; DNA which includes substitutions or differences and encodes the same amino acid sequence as a DNA whose sequence is provided herein or includes substitutions which do not alter the ability of a DNA probe or primer which hybridizes to DNA whose sequence is presented herein to hybridize to the DNA containing the substitutions or differences. It further relates to DNA which encodes a protein or peptide whose sequence is presented herein. The present invention also includes the complements of the DNA sequences presented herein, DNA which hybridizes under stringent (high stringency) conditions to the DNA whose sequences are presented and to RNA transcripts. The invention further relates to encoded proteins, peptides and other products (e.g., glycoproteins) and antibodies which are raised against or bind to proteins or peptides whose amino acid sequences are presented herein or are encoded by DNA whose sequences are provided. As used herein, the term isolated DNA which occurs on the non-recombining region of the human Y chromosome refers to DNA

which has been obtained or removed from the human Y chromosome or DNA, produced by any means (e.g., recombinant techniques, synthetic methods), which has the sequence of such Y chromosome DNA. For example, isolated testis-specific DNA or isolated testis-specific DNA which occurs on the non-recombining region of the human Y chromosome is DNA which has been obtained or removed from the non-recombining region of the human Y chromosome or which has the sequence of such DNA and has been obtained or produced by any means.

Thus, this invention has application to several areas. It may be used diagnostically to identify males with reduced sperm count in whom a gene has been deleted or altered. It may also be used therapeutically in gene therapy treatments to remedy fertility disorders associated with deletion or alteration of a gene described. In one embodiment of a gene therapy method, a gene described herein, or a gene portion which encodes a functional protein, is introduced into a man whose sperm count is reduced and in whom the gene is expressed and the encoded protein replaces the protein normally produced or enhances the quantity produced. The present invention may also be useful in designing or identifying agents which function as a male contraceptive by inducing reduced sperm count. This invention also has application as a research tool, as the nucleotide sequences described herein have been localized to regions of the Y chromosome.

The present invention includes nucleotide sequences described herein, and their complements, which are useful as hybridization probes or primers for an amplification method, such as polymerase chain reaction (PCR), to show the presence, absence or disruption of the gene of the present invention. Probes and primers can have all or a portion of the nucleotide sequence (nucleic acid sequence) of a gene described herein or all or a portion of its

complement. For example, sequences shown in the Figures or Example 2 (SEQ ID NOS.: 1-84), as well as the complements thereof, can be used. The probes and primers can be any length, provided that they are of sufficient length and appropriate composition (appropriate nucleotide sequence) to hybridize to all or an identifying or characteristic portion of the gene described or to a disrupted form of the gene, and remain hybridized under the conditions use. Useful probes include, but are not limited to, nucleotide sequences which distinguish between a gene described herein and an altered form of that gene shown to be associated with reduced sperm count (azoospermia, oligospermia). Generally, the probe will be at least 7 nucleotides, while the upper limit is the length of the gene itself, e.g., up to about 40,000 nucleotides in length. Probes can be, for example, 10 to 14 nucleotides or longer (e.g., 20, 30, 50, 100, 250 nucleotides or any other useful length); the length of a specific probe will be determined by the assay in which it is used.

In one embodiment, the present invention is a method of diagnosing or aiding in the diagnosis of reduced sperm count associated with deletion or alteration of a gene described herein. Any man may be assessed with this method of diagnosis. In general, the man will have been at least preliminarily assessed, by another method, as having a reduced sperm count. By combining nucleic acid probes derived either from the isolated native sequence or cDNA sequence of the gene, or from appropriate primers, with the DNA from a sample to be assessed, under conditions suitable for hybridization of the probes with unaltered complementary nucleotide sequences in the sample but not with altered complementary nucleotide sequences, it can be determined whether the man possesses the intact gene. If the gene is unaltered, it may be concluded that the alteration of the gene is not responsible for the reduced

sperm count. This invention may also be used in a similar method wherein the hybridization conditions are such that the probes will hybridize only with altered DNA and not with unaltered sequences. The hybridized DNA can also be isolated and sequenced to determine the precise nature of the alteration associated with the reduced sperm count. DNA assessed by the present method can be obtained from a variety of tissues and body fluids, such as blood or semen. In one embodiment, the above methods are carried out on DNA obtained from a blood sample.

The invention also provides expression vectors containing a nucleotide (nucleic acid) sequence described herein, which is operably linked to at least one regulatory sequence. "Operably linked" is intended to mean that the nucleotide sequence is linked to a regulatory sequence in a manner which allows expression of the nucleotide sequence. The term "regulatory sequence" included promoters, enhancers, and other expression control elements (see, e.g., Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the protein or peptide desired to be expressed. For instance, the peptides of the present invention can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells or both (see, for example, Broach, *et al.*, Experimental Manipulation of Gene Expression, ed. M, Inouye (Academic Press, 1983) p. 83; Molecular Cloning: A Laboratory Manual, 2nd Ed., ed. Sambrook *et al.* (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17).

Prokaryotic and eukaryotic host cells transfected by the described vectors are also provided by this invention. For instance, cells which can be transfected with the

vectors of the present invention include, but are not limited to, bacterial cells such as *E. coli*, insect cells (baculovirus), yeast and mammalian cells, such as Chinese hamster ovary cells (CHO).

Thus, a nucleotide sequence described herein can be used to produce a recombinant form of the protein via microbial or eukaryotic cellular processes. Production of a recombinant form of the protein can be carried out using known techniques, such as by ligating the oligonucleotide sequence into a DNA or RNA construct, such as an expression vector, and transforming or transfecting the construct into host cells, either eukaryotic (yeast, avian, insect or mammalian) or prokaryotic (bacterial cells). Similar procedures, or modifications thereof, can be employed to prepare recombinant proteins according to the present invention by microbial means or tissue-culture technology.

The present invention also pertains to pharmaceutical compositions comprising the proteins and peptides described herein. For instance, the peptides or proteins of the present invention can be formulated with a physiologically acceptable medium to prepare a pharmaceutical composition. The particular physiological medium may include, but is not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polethylene glycol) and dextrose solutions. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to medicinal chemists, and will depend on the ultimate pharmaceutical formulation desired. Methods of introduction of exogenous polypeptides at the site of treatment include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral and intranasal. Other suitable methods of introduction can also include rechargeable or biodegradable devices and slow release polymeric devices. The

pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

This invention also has utility in methods of treating disorders of reduced sperm count associated with deletion or alteration of a gene described herein. These genes may be used in a method of gene therapy, whereby the gene or a gene portion encoding a functional protein is inserted into cells in which the functional protein is expressed and from which it is generally secreted to remedy the deficiency caused by the defect in the native gene.

The present invention is also related to antibodies which bind a protein or peptide encoded by all or a portion of a gene of the present invention, as well as antibodies which bind the protein or peptide encoded by all or a portion of a disrupted form of the gene. For instance, polyclonal and monoclonal antibodies which bind to the described polypeptide or protein are within the scope of the invention. A mammal, such as a mouse, hamster or rabbit, can be immunized with an immunogenic form of the protein or peptide (an antigenic fragment of the protein or peptide which is capable of eliciting an antibody response). Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques are well known in the art. The protein or peptide can be administered in the presence of an adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibody.

Following immunization, anti-peptide antisera can be obtained, and if desired, polyclonal antibodies can be isolated from the serum. Monoclonal antibodies can be isolated from the serum. Monoclonal antibodies can also be produced by standard techniques which are well known in the

art (Koehler and Milstein, Nature 256: 495-497 (19775); Kozbar *et al.*, Immunology Today 4: 72 (1983); and Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)). Such antibodies are useful as diagnostics for the intact or disrupted gene and also as research tools for identifying either the intact or disrupted gene.

The present invention is illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLE 1 ISOLATION OF CDNA CLONES FROM HUMAN TESTIS LIBRARY

"cDNA selection" (M. Lovett *et al.*, *Proc. Natl. Acad. Sci. USA 88:*9628 (1991)) was carried out using bulk cDNA prepared from human adult testes (Clontech, Palo Alto, CA) and, as selector, a cosmid library prepared from flow-sorted Y chromosomes (Lawrence Livermore National Laboratory: LL0YNC03). A total of 3600 random cosmids, providing nearly five-fold coverage of the 30-Mb euchromatic region, were used to generate 150 pools of selector DNA. Using each of the 150 selector pools, we carried out four successive rounds of cDNA selection, followed by two rounds of subtraction with human COT-1 DNA (Gibco BRL, Gaithersburg, MD) to remove highly repetitive sequences. A plasmid library was prepared from each of the 150 resulting pools of selected cDNA fragments, and 24 clones from each library were sequenced from one end. Of the 3600 sequences generated, about 600 were of poor technical quality and about 500 were found to derive from cloning vector or *E. coli* host, leaving 2539 sequences for further analysis. Of the 2539 sequence fragments, 536 corresponded to previously reported NRY genes (487 to *TSPY*, 15 to *YRRM*, 14 to *RPS4Y*, 9 to *SMCY*, 5 to *DAZ*, 3 to *SRY*, 3 to *ZFY*) and 41 corresponded to previously reported pseudoautosomal genes (15 to *XE7*, 11 to *CSF2RA*, 4 to *IL3RA*,

3 to *ASMT*, 3 to *IL9R*, 2 to *ANT3*, 2 to *MIC2*, 1 to *SYBL1*). Electronic analysis of the roughly 2000 remaining sequences revealed that about 200 contained known repetitive elements, and these were not pursued. By electronically identifying redundancies and sequence overlaps, the remaining sequences were reduced to 1093 sequence contigs. Sequences representing these 1093 contigs were individually hybridized to dot-blotted yeast genomic DNAs of 60 YACs comprising most of the Y's euchromatic region (S. Foote *et al.*, *Science 258:*60 (1992)). 181 sequences that hybridized to the great majority of the YACs were judged likely to contain highly repeated elements and were not pursued, leaving 912 sequences for further analysis. The 912 sequences were individually hybridized to Southern blots of R1-digested human 46,XX female and 49,XYYYY male (L. Sirota *et al.*, *Clin. Genet. 19:*87 (1981)) genomic DNAs. Blots were hybridized at 65°C in Church's buffer (0.5 M Na_2PO_4 at pH7.5, with 7% SDS), and washed at 65°C in 1X SSC and 0.1% SDS, with 832 hybridizations yielding interpretable results. Many sequences appeared to contain highly repeated elements common to males and females, or failed to detect an unambiguously Y-specific restriction fragment, and these were not pursued. By contrast, 308 sequences hybridized to at least one prominent fragment present in 49,XYYYY but absent in 46,XX, suggesting that these sequences derived from the NRY. Each of these 308 sequences was individually used to screen, by hybridization, about 2 million plaques from a 1 phage library of human adult testis cDNA (Clontech, Palo Alto, CA).

EXAMPLE 2 LOCALIZATION OF 12 NOVEL GENES ON THE Y CHROMOSOME

Genes were localized on a previously reported NRY deletion map by testing with PCR for their presence or

absence in individuals carrying partial Y chromosomes (D. Vollrath *et al.*, *Science 258*:52 (1992)). Most genes were localized to a single deletion interval. Some genes could not be unambiguously placed because copies exist in multiple locations in the NRY. In such cases, genes were localized by PCR testing of YACs encompassing the NRY's euchromatic region (S. Foote *et al.*, *Science 258*:60 (1992)). X homologs of Y genes were mapped onto the X by PCR testing a panel of human/rodent somatic hybrid cell lines (Research Genetics, Huntsville, AL). All PCR assays consists of 30 cycles of the following conditions: 1 min denaturing at 94°C, 45 sec annealing at 60°C, and 45 sec extension at 72°C. *TB4X* primers were designed from an unreported intron. TPRX primers were designed from unreported cDNA sequence. All other primers were designed from cDNA sequences as submitted to Genbank. PCR primers were as follows:

GENE	LEFT PRIMER	RIGHT PRIMER
DBY	CATTCGGTTTTACCAGCCAG	CAGTGACTCGAGGTTCAATG
	(SEQ ID NO.: 51)	(SEQ ID NO.: 52)
TPRY	GCATCATAATATGGATCTAGTAGG	GGAGATACTGAATAGCATAGC
	(SEQ ID NO.: 53)	(SEQ ID NO.: 54)
TB4Y	CAAAGACCTGCTGACAATGG	CTCCGCTAAGTCTTTCACC
	(SEQ ID NO.: 55)	(SEQ ID NO.: 56)
EIF1AY	CTCTGTAGCCAGCCTCTTC	GACTCCTTTCTGGCGGTTAC
	(SEQ ID NO.: 570	(SEQ ID NO.: 58)
DFFRY	GAGCCCATCTTTGTCAGTTTAC	CTGCCAATTTTCCACATCAACC
	(SEQ ID NO.: 59)	(SEQ ID NO.: 60)
CDY	GGCTCAAAATCCACTGACG	CAAGCGATATCTCACCACC
	(SEQ ID NO.: 61)	(SEQ ID NO.: 62)
BPY1	CTCCCTGAGCAGCAACTAAG	GTCATCAACATGGGAAGCAC
	(SEQ ID NO.: 63)	(SEQ ID NO.: 64)
BPY2	CCAGGACCATGTGATATGG	CTAATTCCCTCTTTACGCATGACC
	(SEQ ID NO.: 65)	(SEQ ID NO.: 66)

XKRY	CACTCATGGAGAAGGGTAGG (SEQ ID NO.: 67)	GTCACACTCAGCCTCTTTAC (SEQ ID NO.: 68)
PTPRY	GAGCACACCACACCAGAAAC (SEQ ID NO.: 69)	CTCAGACTGACCTCGGACTG (SEQ ID NO.: 70)
TTY1	CTCTGGGAATCAAATTCGAGG (SEQ ID NO.: 71)	GTCTTTCAGCCAATCCAAGG (SEQ ID NO.: 72)
TTY2	GACAACTCTGACAGCCAGG (SEQ ID NO.: 73)	GTCAGAACTCCCAAACAGG (SEQ ID NO.: 74)
DBX	CTACATGCAGATGACATGGTG (SEQ ID NO.: 75)	GGCCAAGGTGCATAGGTG (SEQ ID NO.: 76)
TPRX	CATGTTCCCTGTAGCACATC (SEQ ID NO.: 77)	CGTTTCCATTACTTCCATTTCCTG (SEQ ID NO.: 78)
TB4X	CCCGCCCTTTCATCATCC (SEQ ID NO.: 79)	GCTCCCCAAAGTAGCCTTC (SEQ ID NO.: 80)
EIF1AX	CACGAGGCGCCATTTGCTG (SEQ ID NO.: 81)	CTGGAGGCCAGGCAACGTG (SEQ ID NO.: 82)
DFFRX	CCTCCACCTGAAGATGCC (SEQ ID NO.: 83)	CTGAGATCCAGGTGAATGG (SEQ ID NO.: 84)

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

We claim:

1. Isolated testis-specific DNA which occurs on the non-recombining region of the human Y chromosome or the complement thereof.

2. The isolated testis-specific DNA of Claim 1 which occurs in multiple copies on the non-recombining region of the human Y chromosome or the complement thereof.

3. The isolated testis-specific DNA of Claim 2 selected from the group consisting of:

(a) a CDY gene or a characteristic portion thereof;
(b) a BPY 1 gene or a characteristic portion thereof;
(c) a BPY 2 gene or a characteristic portion thereof;
(d) an XKRY gene or a characteristic portion thereof;
(e) a PTPRY gene or a characteristic portion thereof;
(f) TTY1 DNA; or a characteristic portion thereof;
(g) TTY 2 DNA; or a characteristic portion thereof;
(h) a complement of (a);
(i) a complement of (b);
(j) a complement of (c);
(k) a complement of (d);
(l) a complement of (e);
(m) a complement of (f);
(n) a complement of (g);
(o) DNA encoding the amino acid sequence of SEQ ID No.: 39;.
(p) DNA encoding the amino acid sequence of SEQ ID No.: 40;
(q) DNA encoding the amino acid sequence of SEQ ID No.: 42;
(r) DNA encoding the amino acid sequence of SEQ ID No.: 44;

(s) DNA encoding the amino acid sequence of SEQ ID No.: 46;

(t) DNA encoding the amino acid sequence of SEQ ID No.: 48; and

(u) DNA which hybridizes to a DNA of any one of(a) through (t) under stringent conditions.

4. Isolated testis specific DNA selected from the group consisting of:

(a) DNA of SEQ ID No.: 37;

(b) DNA of SEQ ID No.: 38;

(c) DNA of SEQ ID No.: 41;

(d) DNA of SEQ ID No.: 43;

(e) DNA of SEQ ID No.: 45;

(f) DNA of SEQ ID No.: 47;

(g) DNA of SEQ ID No.: 49;

(h) DNA of SEQ ID No.: 50;

(i) DNA encoding the amino acid sequence of SEQ ID No.39;

(j) DNA encoding the amino acid sequence of SEQ ID No.40;

(k) DNA encoding the amino acid sequence of SEQ ID No.42;

(l) DNA encoding the amino acid sequence of SEQ ID No.44;

(m) DNA encoding the amino acid sequence of SEQ ID No.46;

(n) DNA encoding the amino acid sequence of SEQ ID No.48;

(o) a complement of a DNA of any one of (a) through (n); and

(p) DNA which hybridizes to a DNA of any one of (a) through (o) under stringent conditions.

5. Isolated X-homologous DNA which occurs on the non-recombining region of the human Y chromosome, is not testis-specific and has a homolog on the human X chromosome.

6. The isolated DNA of Claim 5 selected from the group consisting of:

(a a DBY gene or a characteristic portion thereof;
(b) a TPRY gene or a characteristic portion thereof;
(c) a TB4Y gene or a characteristic portion thereof;
(d) an EIF1AY gene or a characteristic portion thereof;
(e) a DFFRY gene or a characteristic portion thereof;
(f) a complement of (a);
(g) a complement of (b);
(h) a complement of (c);
(i) a complement of (d);
(j) a complement of (e);
(k) a complement of (f);
(l) DNA encoding the amino acid sequence of SEQ ID No.: 18;
(m) DNA encoding the amino acid sequence of SEQ ID No.: 22;
(n) DNA encoding the amino acid sequence of SEQ ID No.: 23
(o) DNA encoding the amino acid sequence of SEQ ID No.: 24;
(p) DNA encoding the amino acid sequence of SEQ ID No.: 28;
(q) DNA encoding the amino acid sequence of SEQ ID No.: 32;
(r) DNA encoding the amino acid sequence of SEQ ID No.: 36; and;

(s) DNA which hybridizes to a DNA of any one of (a) through (r) under stringent conditions.

7. Isolated X-homologous human DNA selected from the group consisting of:

(a) DNA of SEQ ID No.: 17 or a characteristic portion thereof;

(b) DNA of SEQ ID No.: 19 or a characteristic portion thereof;

(c) DNA of SEQ ID No.: 20 or a characteristic portion thereof;

(d) DNA of SEQ ID No.: 21 or a characteristic portion thereof;

(e) DNA of SEQ ID No.: 26 or a characteristic portion thereof;

(f) DNA of SEQ ID No.: 30 or a characteristic portion thereof;

(g) DNA of SEQ ID No.: 34 or a characteristic portion thereof;

(h) DNA encoding the amino acid sequence of SEQ ID No.: 18;

(i) DNA encoding the amino acid sequence of SEQ ID No.: 22;

(j) DNA encoding the amino acid sequence of SEQ ID No.: 23;

(k) DNA encoding the amino acid sequence of SEQ ID No.: 24;

(l) DNA encoding the amino acid sequence of SEQ ID No.: 28;

(m) DNA encoding the amino acid sequence of SEQ ID No.: 32;

(n) DNA encoding the amino acid sequence of SEQ ID No.: 36;

(o) a complement of a DNA of any one of (a) through (n); and

(p) DNA which hybridizes to a DNA any one of (a) through (o) under stringent conditions.

8. A DNA probe comprising all or a characteristic portion of DNA of Claim 4.

9. A DNA probe comprising all or a characteristic portion of DNA of Claim 7.

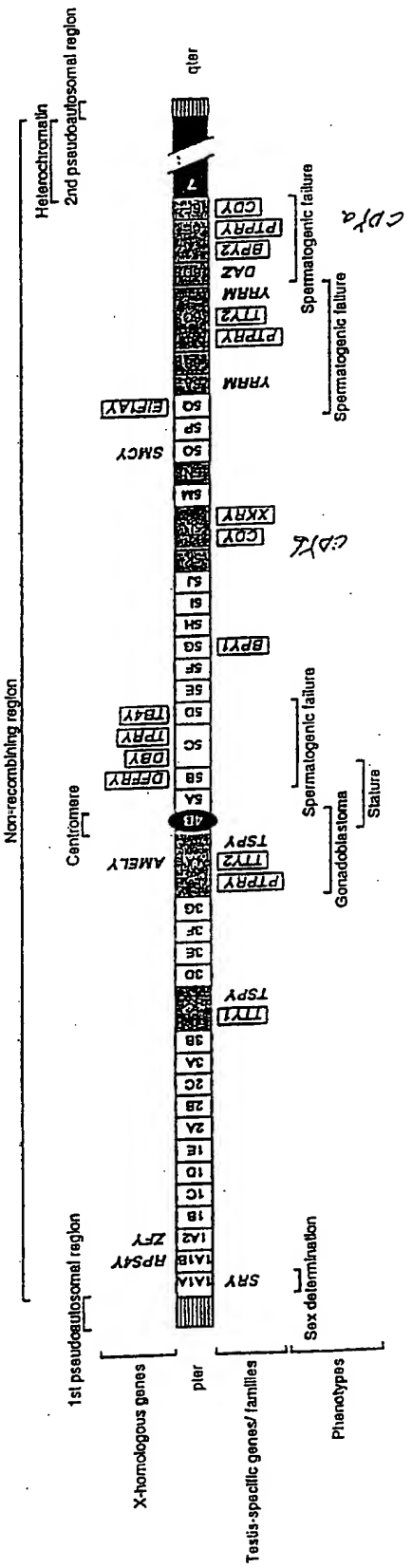

FIG. 1

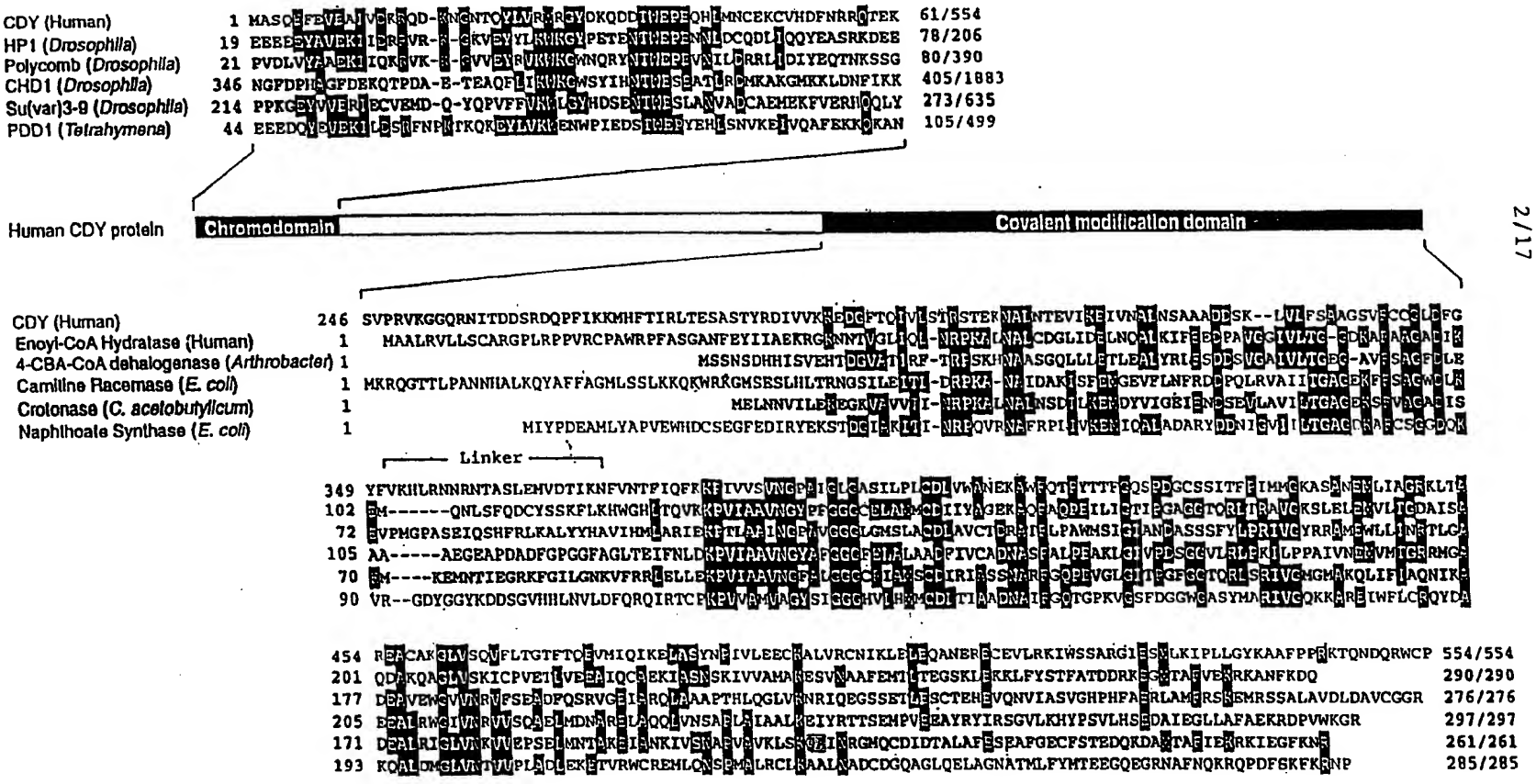

FIG. 2

DBX & *DBY*
long and short transcripts

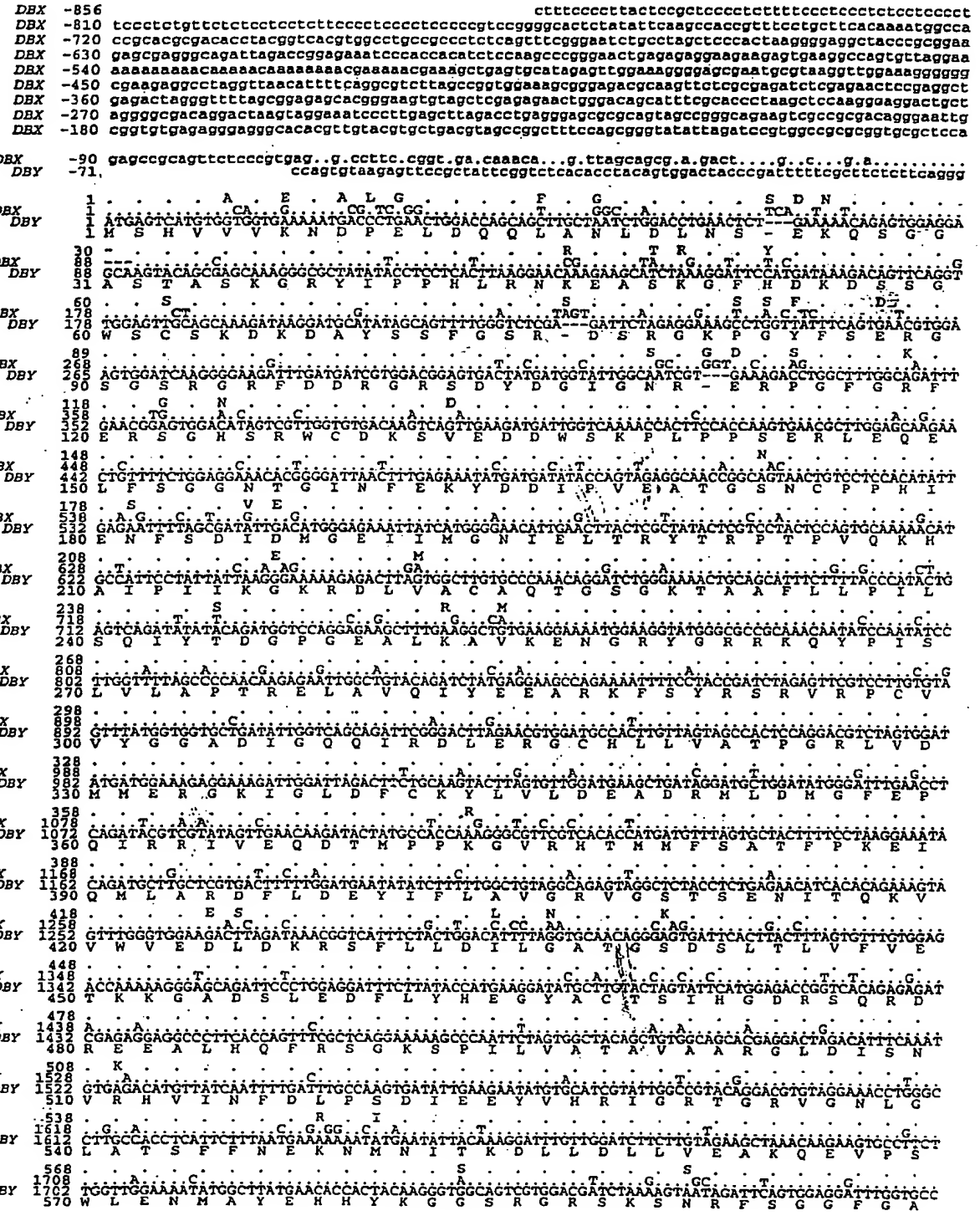

FIG. 3A

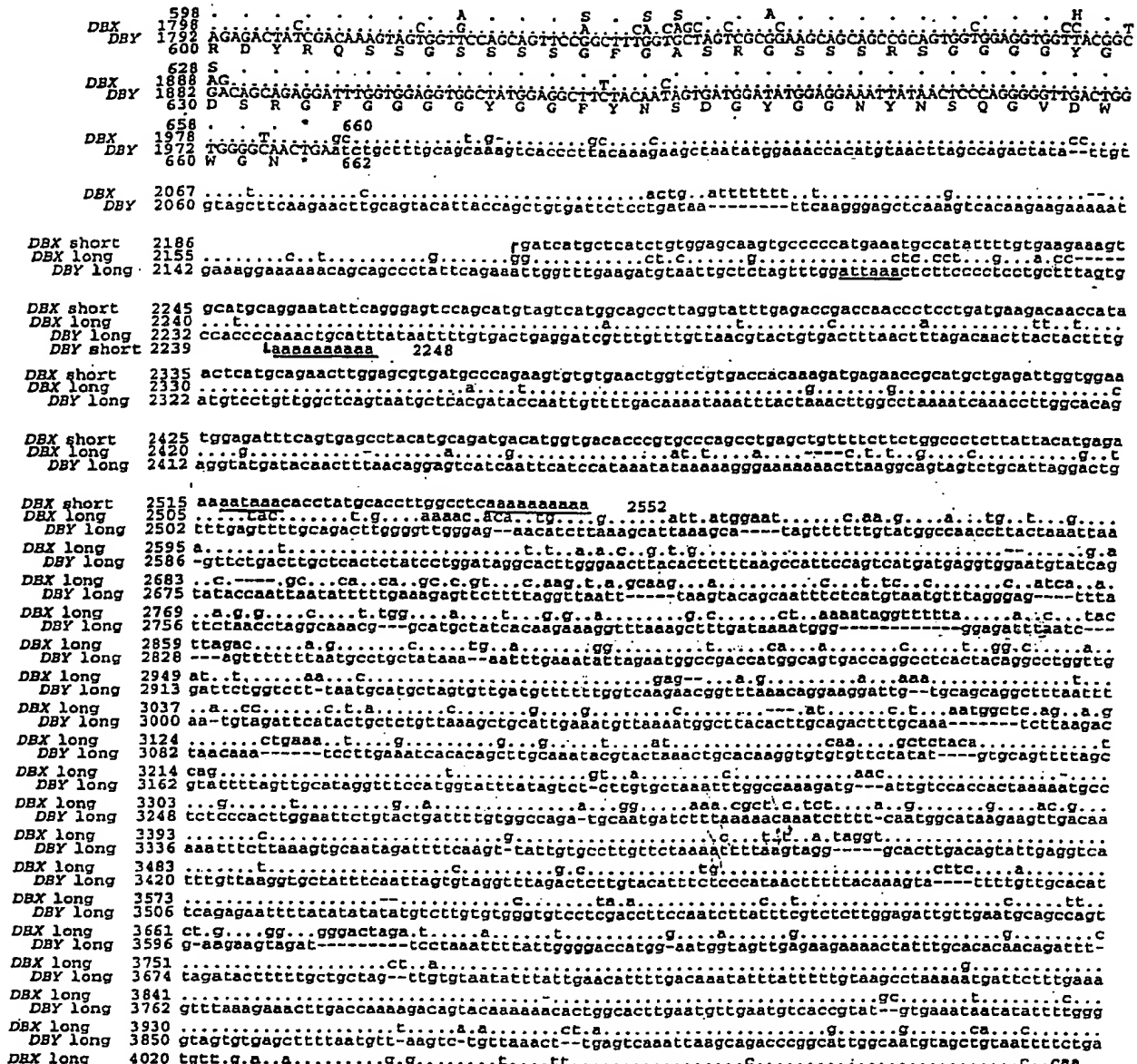

FIG. 3B

TPRY
short, medium and long transcripts

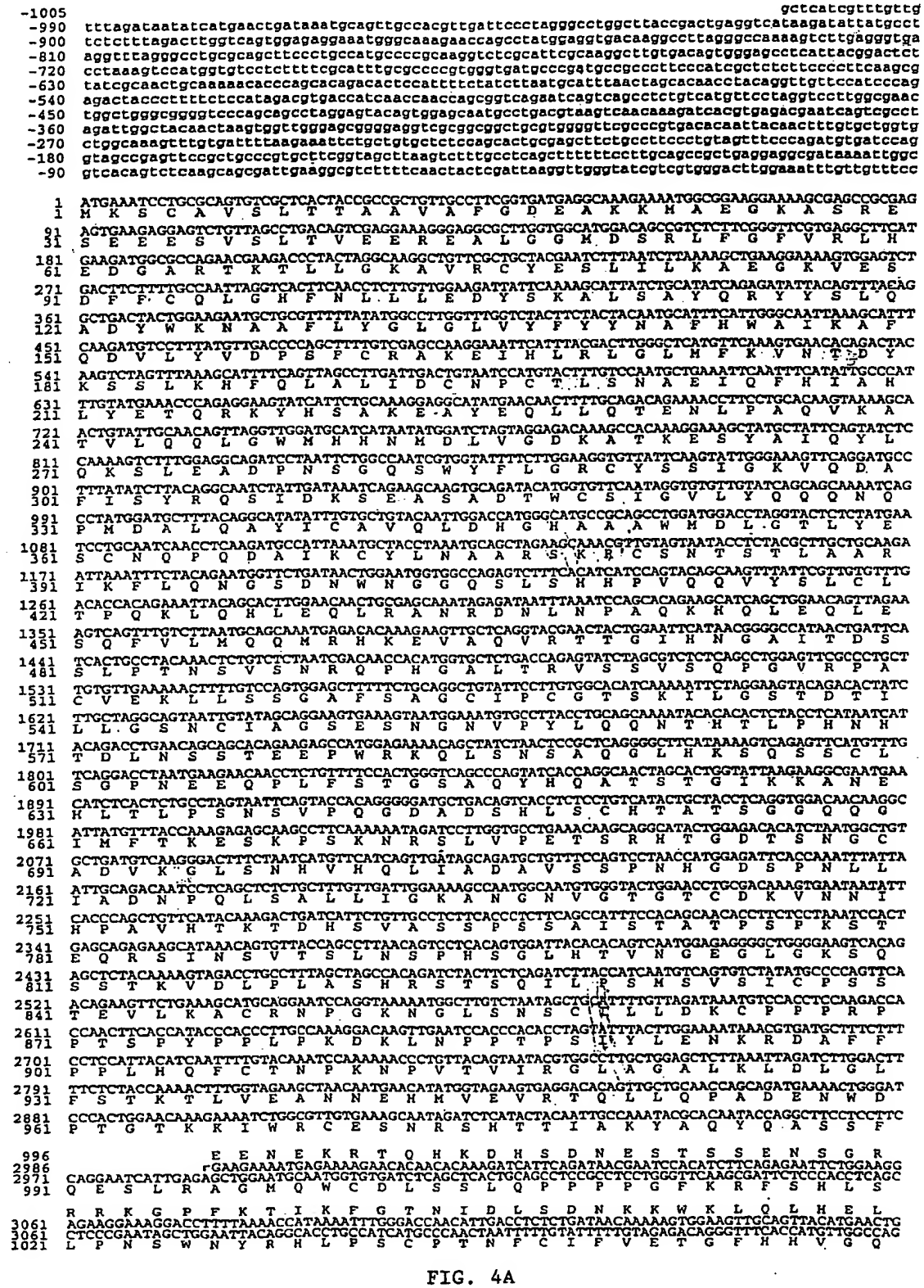

FIG. 4A

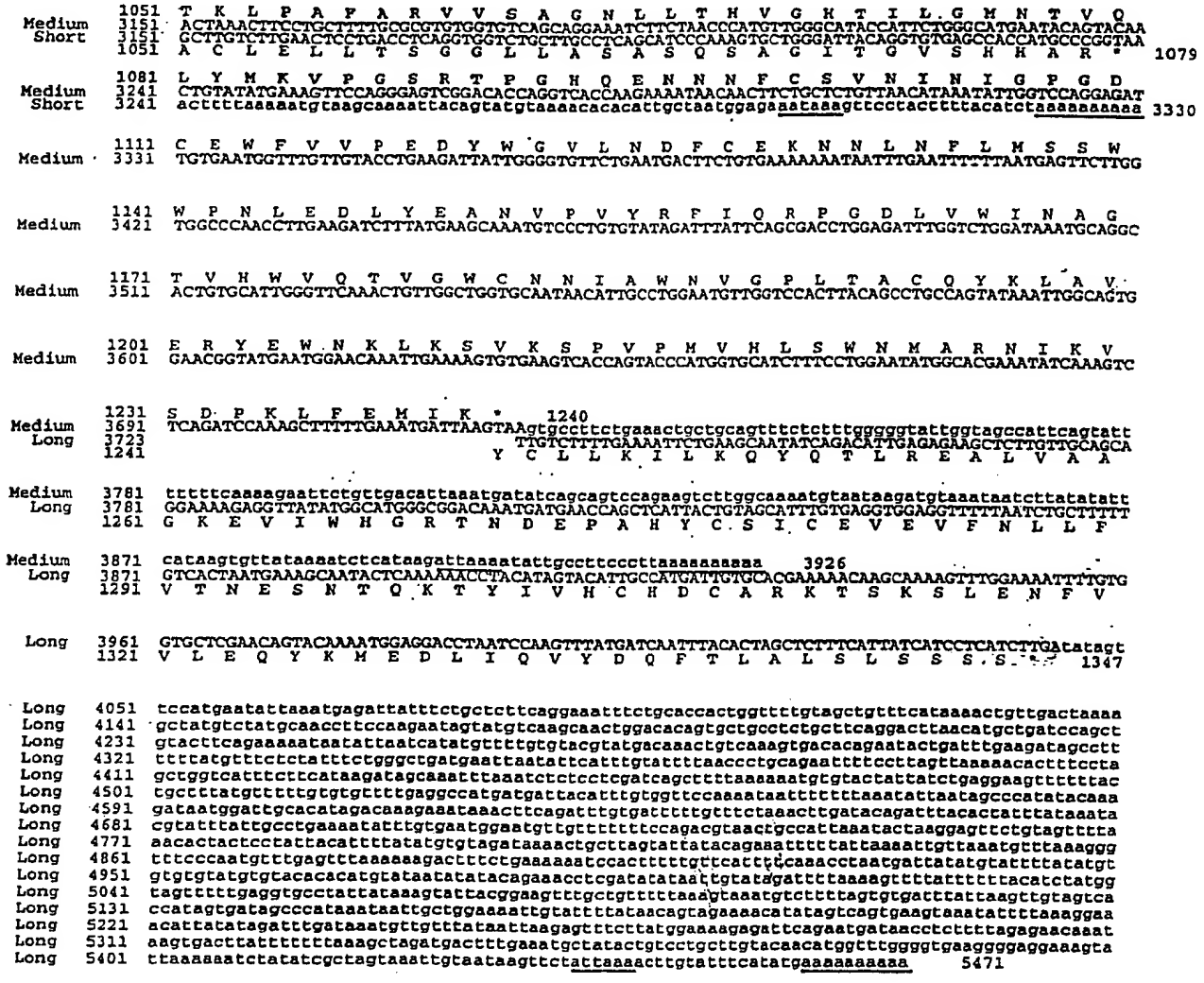

FIG. 4B

TB4X & TB4Y

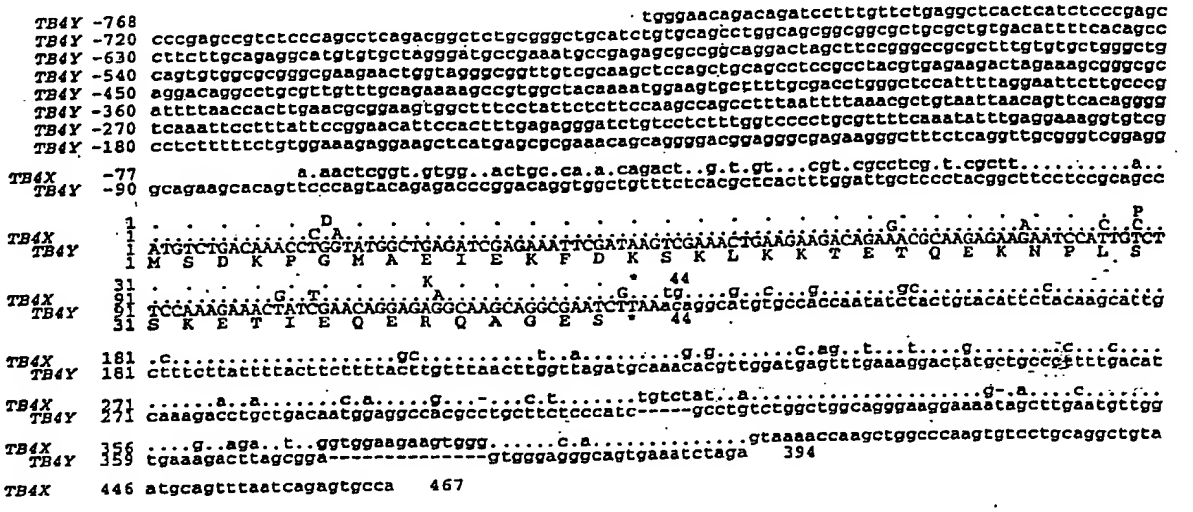

FIG. 5

EIF1AX & EIF1AY

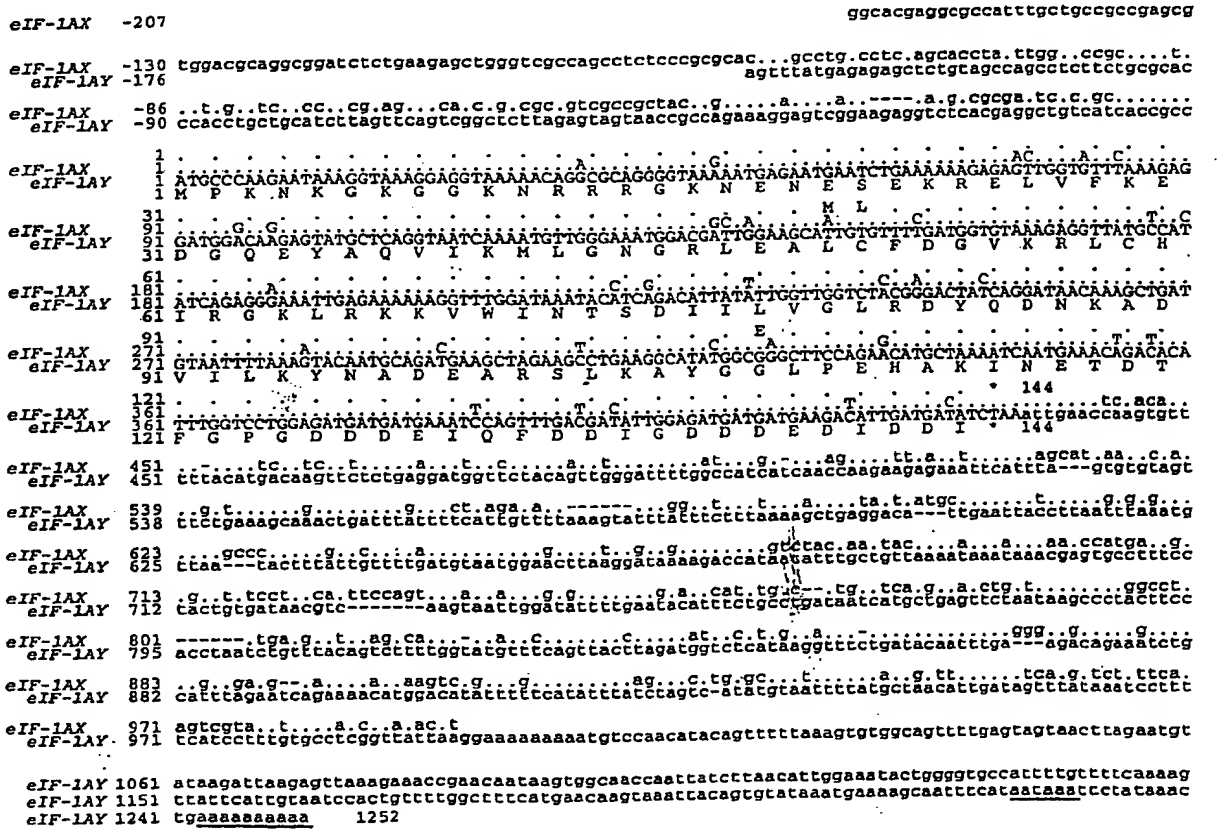

FIG. 6

DFFRX & DFFRY

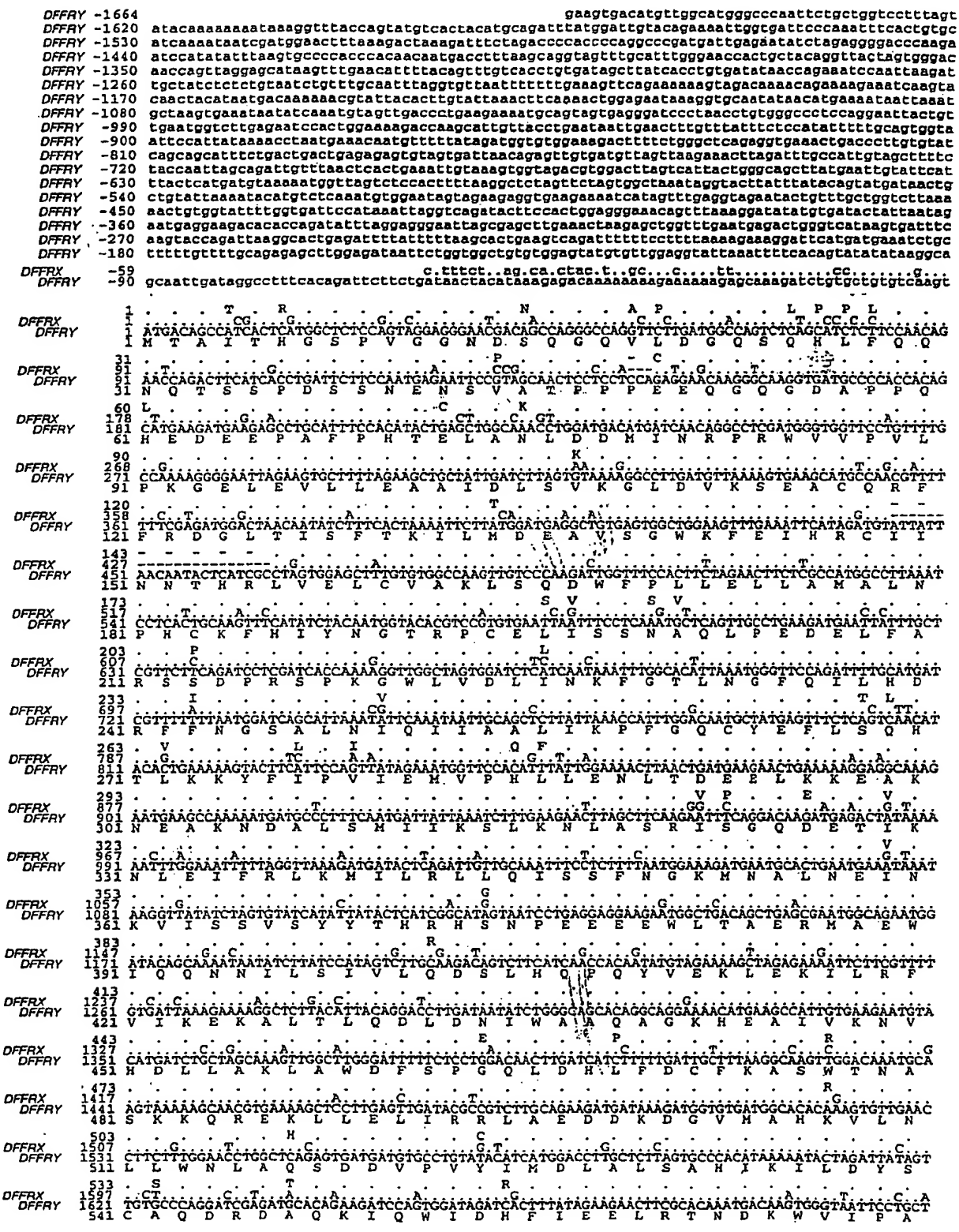

FIG. 7A

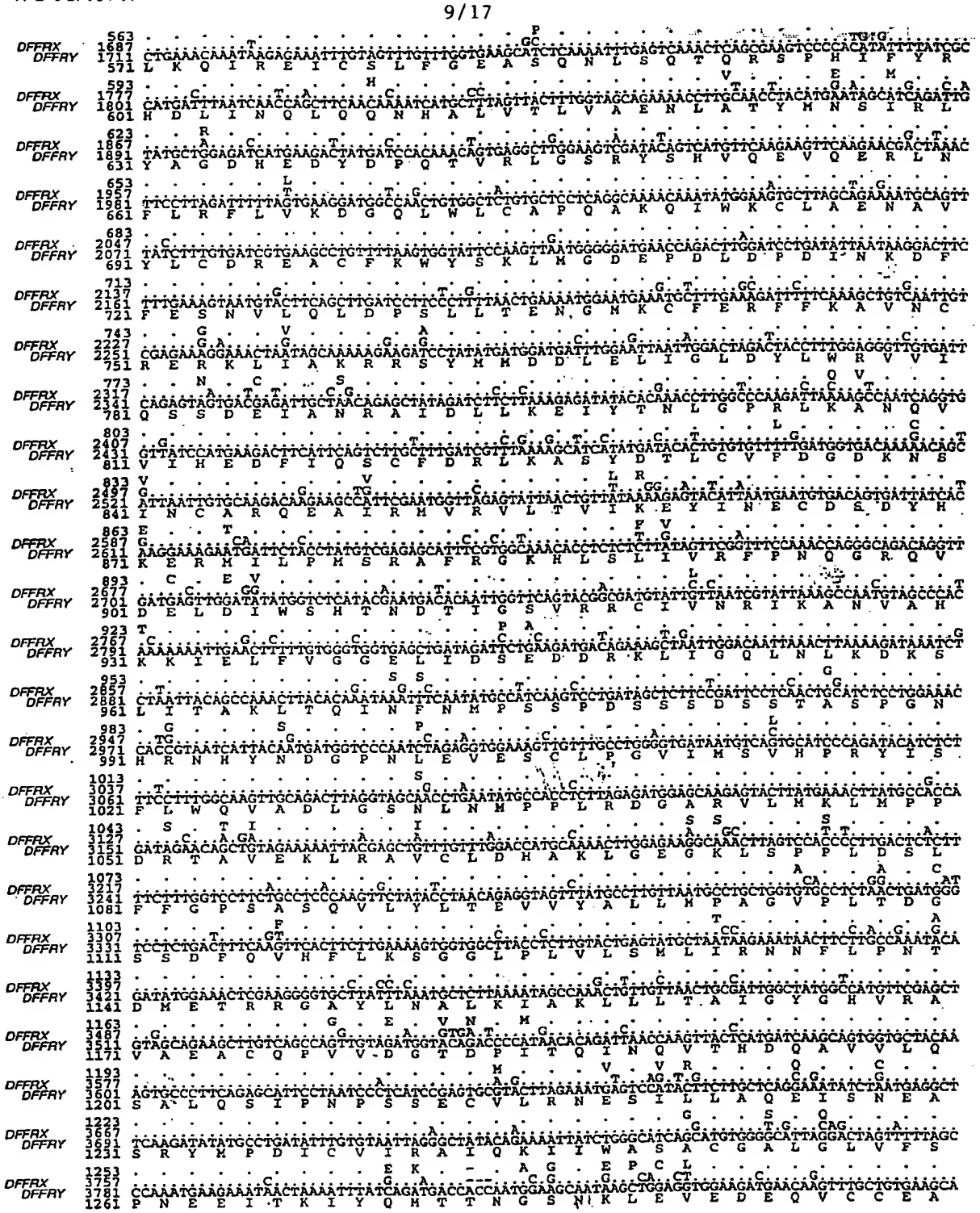

FIG. 7B

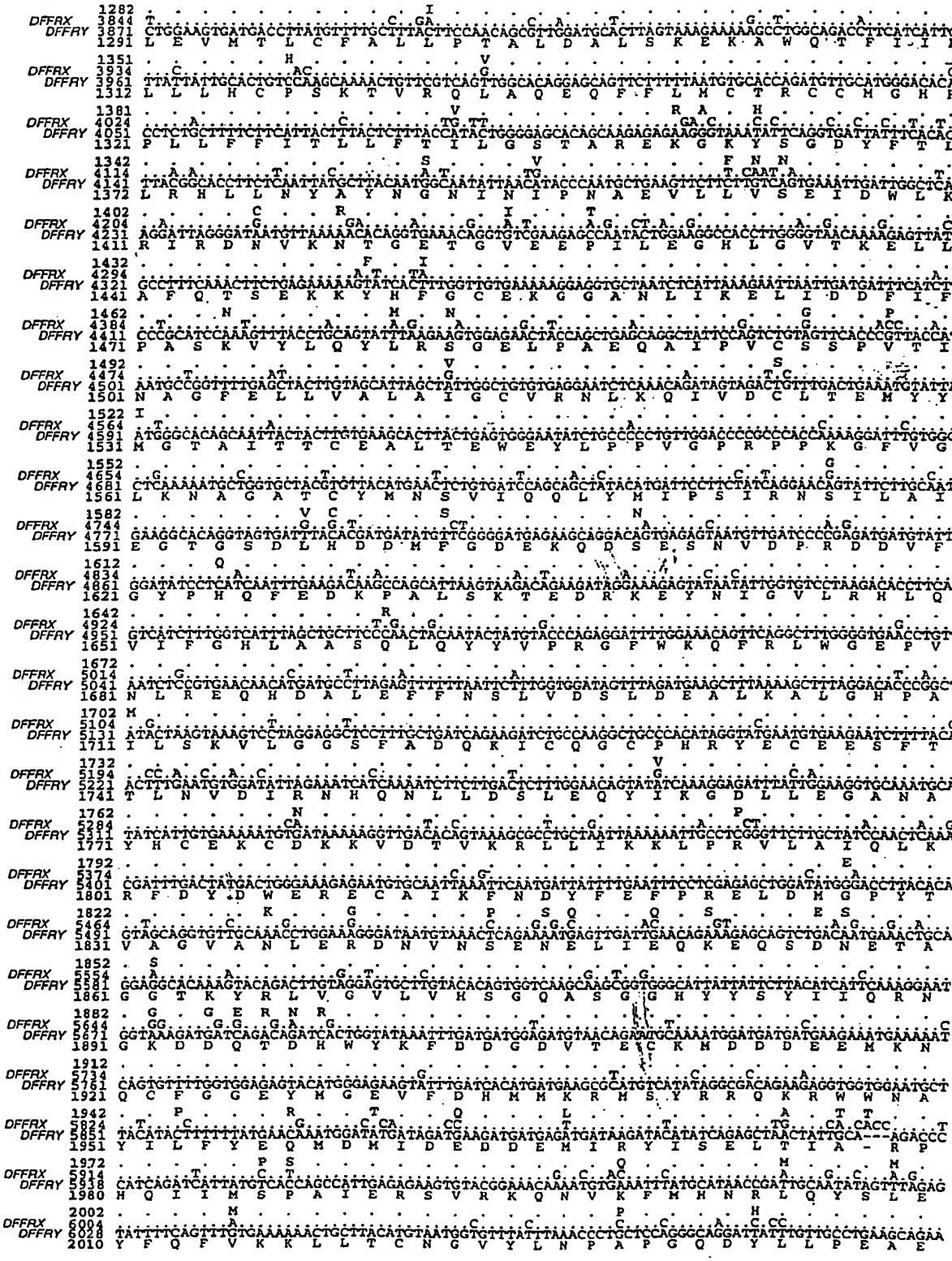

FIG. 7C

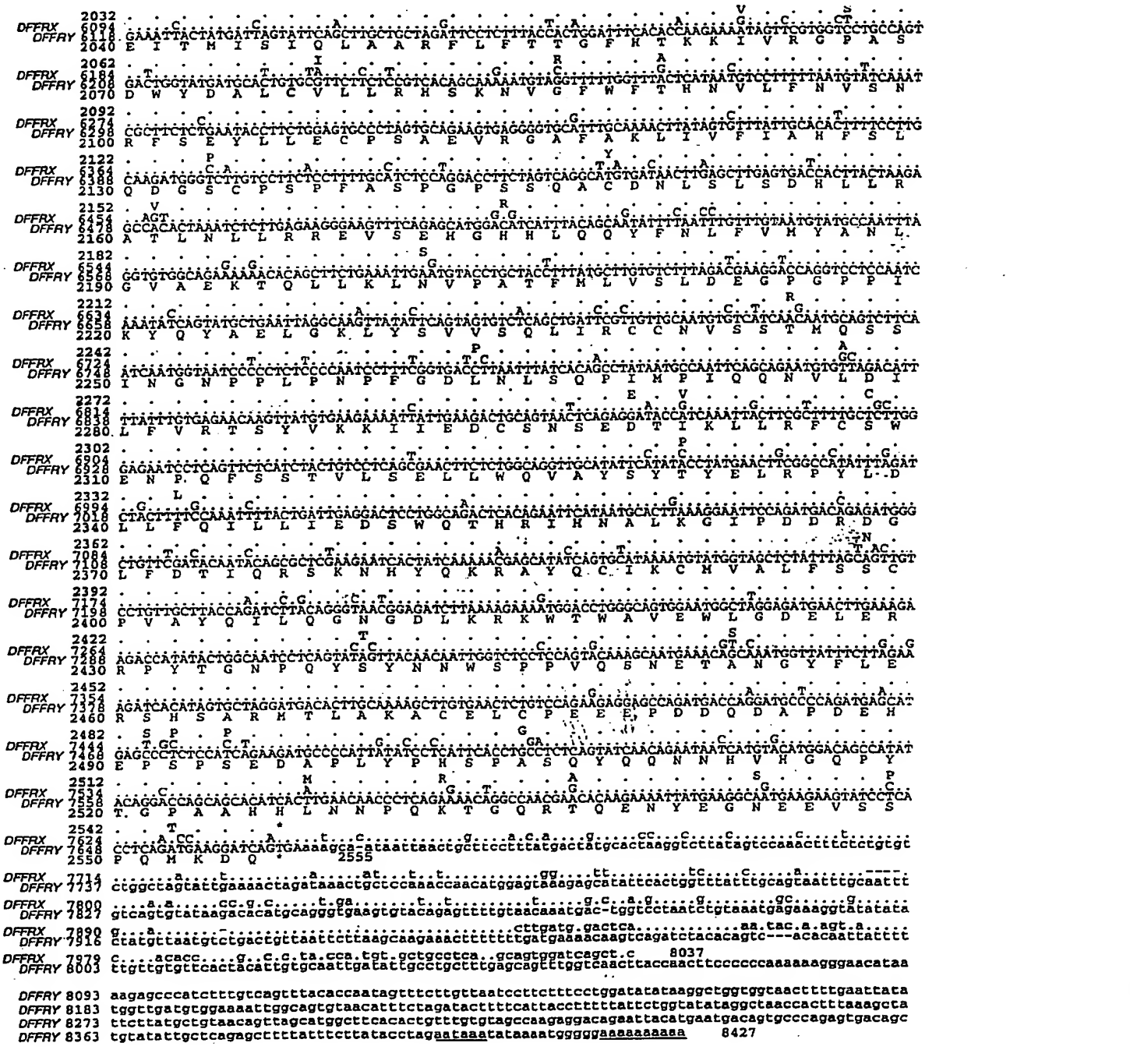

FIG. 7D

CDYa & CDYb

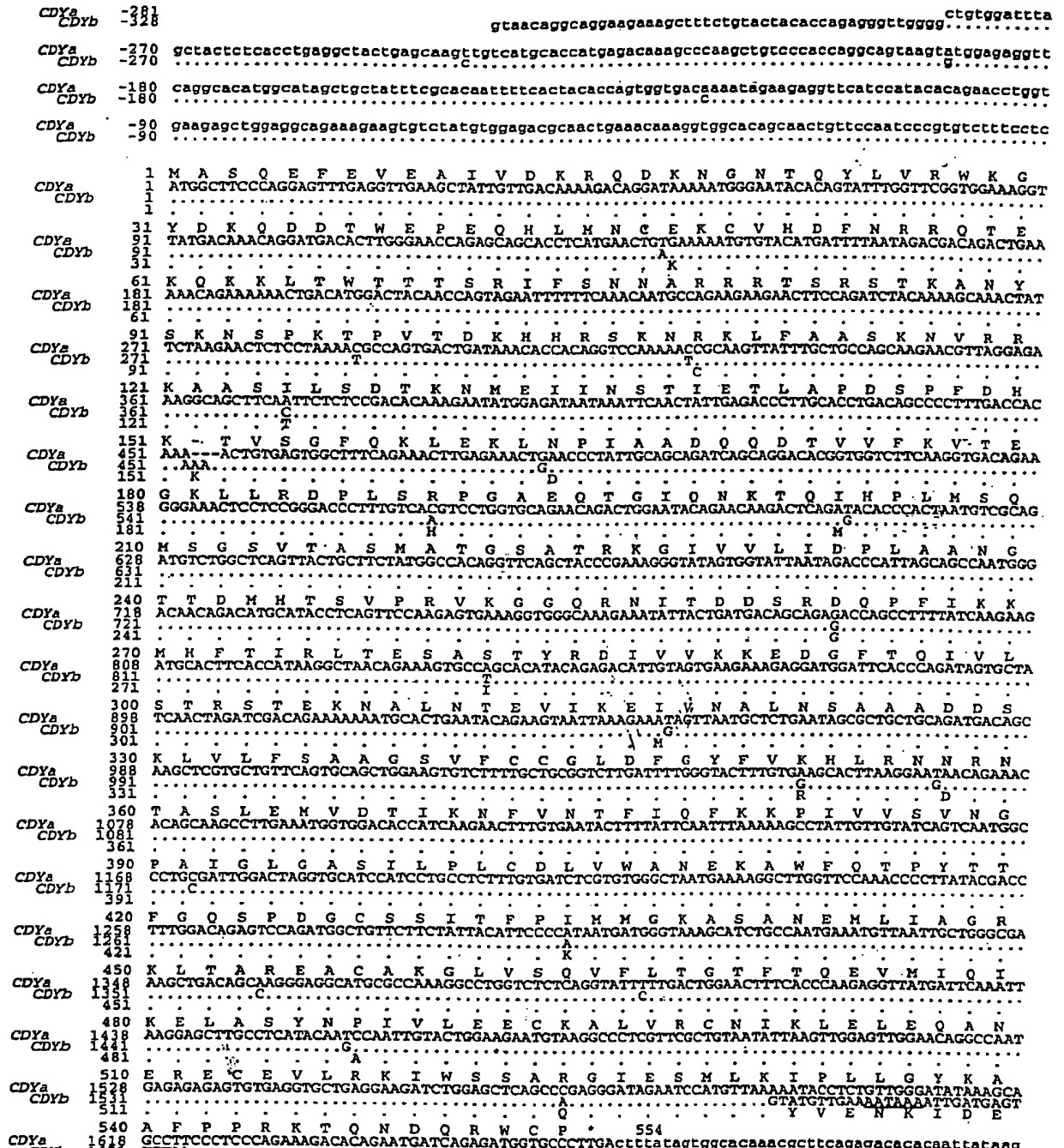

FIG. 8

BPY1

```
-72                                  gagaggggtatacacagggaggccaggcagcctggagttagtcgaccgttgcgagacgttgagctgcggcag

  1  ATGAGTCCAAAGCCGAGAGCCTCGGGACCTCCGGCCAAGGCCAAGGAGACAGGAAAGAGGAAGTCCTCCTCTCAGCCGAGCCCCAGTGGC
  1  M  S  P  K  P  R  A  S  G  P  P  A  K  A  K  E  T  G  K  R  K  S  S  S  Q  P  S  P  S  G
 91  CCGAAGAAGAAGACTACCAAGGTGGCCGAGAAGGGAGAAGCAGTTCGTGGAGGGAGACGCGGGAAGAAAGGGGCTGCGACAAAGATGGCG
 31  P  K  K  K  T  T  K  V  A  E  K  G  E  A  V  R  G  G  R  R  G  K  K  G  A  A  T  K  M  A
181  GCCGTGACGGCACCTGAGGCGGAGAGCGGGCCAGCGGCACCCGGCCCCAGCGACCAGCCCAGCCAGGAGCTCCCTCAGCACGAGCTGCCG
 61  A  V  T  A  P  E  A  E  S  G  P  A  A  P  G  P  S  D  Q  P  S  Q  E  L  P  Q  H  E  L  P
271  CCGGAGGAGCCAGTGAGCGAGGGGACCCAGCACGACCCCCTGAGTCAGGAGAGCGAGCTGGAGGAACCACTGAGTAAGGGGCGCCCATCT
 91  P  E  E  P  V  S  E  G  T  Q  H  D  P  L  S  Q  E  S  E  L  E  E  P  L  S  K  G  R  P  S
361  ACTCCCCTATCTCCCTGAgcagcaactaagtttaggcccagctgccagacctcagagatctcaccagcagggtgcttcccatgttgatga
121  T  P  L  S  P  *   125
451  caataaaatgaatgtgttgcaaaaaaaaaa  480
                        94
```

FIG. 9

BPY2

```
-332                                  aatatctcaggacccaggaccatgtgatatgggcccaacacctggatgatgttactcttctg
-270  cctaggtcatgcgtaaagagggaattagggcatattgcttggcccagtcccgtaatgatatgactctcctgcttgtgccagagccacaga
-180  agtgtgcttggtgacataatcttttgaggctgtcacatcaccaagattatattgtatcactggaccagcataaagctgacacttctgacta
 -90  tgcccagccttcaaataatactacactgtataattggctcaacacccaggtgatattgttccatttacctgagaccagatagaaagccta

  1  ATGATGACGCTTGTCCCCAGAGCCAGGACACGTGCAGGACAGGATCATTACTCTCATCCCTGCCCCAGATTTTCACAGGTGCTGCTTACA
  1  M  M  T  L  V  P  R  A  R  T  R  A  G  Q  D  H  Y  S  H  P  C  P  R  F  S  Q  V  L  L  T
 91  GAGGGCATCATGACATATTGCTTGACAAAGAACCTAAGTGATGTTAATATTCTGCATAGGTTGCTAAAAAATGGGAATGTGAGAAATACC
 31  E  G  I  M  T  Y  C  L  T  K  N  L  S  D  V  N  I  L  H  R  L  L  K  N  G  N  V  R  N  T
181  TTGCTTCAGTCCAAAGTGGGCTTGCTGACATATTATGTGAAACTGTACCCGGGTGAAGTGACTCTTCTGACTAGGCCCAGCATACAAATG
 61  L  L  Q  S  K  V  G  L  L  T  Y  Y  V  K  L  Y  P  G  E  V  T  L  L  T  R  P  S  I  Q  M
271  AGATTATGCTGTATCACTGGCTCAGTGTCGAAGCCCAGATCACAGAAGTAAttgtgccatatgtggaacaagcagctaagcaatagataa
 91  R  L  C  C  I  T  G  S  V  S  K  P  R  S  Q  K  *   106
361  catccatcgtggctctgccttcaaagggaaattttacatatgtcactgggaccatcacccagatgatgtcctgcccactaaaagaattgt
451  gacataacgctgactgcaaaaactgggtaatgcaacctcctcttttattctggagtctgccaaaacaagggattatcacatattgcggag
541  tccagcacccaggtaaaatttttgtcatatacccagcttcagataccatgcaatgatacaactatcatacctggacccaaagaggagagat
631  attttgattctcattgccattcttatggccacaagcaaagtaatggttcttcatagtggtataaagttcacacagtattatgacactccca
721  gcgtatcatagaaaatgtgagtagtacaatgagtgttataacagggaacagcaaacaatgctattgtgattattggattcacacccagc
811  tgacgcgactatcattcttcttcacaagaacagaacctgcaaataaagtactaaatcttcaccaaaaaaaaaa  880
```

FIG. 10

XKRY

```
-663                                                                  attaaaaacttctgataaaattacctaagtaca
-630  cacaaacaaaaacatgcccacacaaatcacttaatttctaaaacttttaattttttctgcttcttctagtaccttgtattccatcacacagc
-540  aaaatctggcagctccacttccagaatttacttgaactccacagcttatttccgatttcctgttatcaccagagtctaaaacacagttta
-450  tattgcattcacctcctatttttacaccgtaatttcctactttacactctaactttatataaaaaagaaactacctttttcaagatctaatt
-360  cacgcaattttatttgttcttaattgagacttcttttctaggtgctgtcacaccttgtaacgtcagatacaaatgtctctatccaatttca
-270  tgagttccagttatttattttaagggaatgtgtatatacatttataaatttgtgtatgtgtgtattcacttattctttatttttatatgt
-180  tttgcatgcatatattcactaaatccctgataatagaaagataacaaatctttttttttctttcttttttgtatgtaaattatttttccga
 -90  aggaggtgggttgggagaaatatatcttaacttggcaagtttaaaagagaaagtggccattactaatgaaaattattctctagcattttc

  1  ATGTTTATCTTTAATAGCATTGCTGATGACATATTCCCTCTTATCAGTTGTGTAGGTGCCATTCACTGCAATATACTGGCCATCCGCACT
  1  M  F  I  F  N  S  I  A  D  D  I  F  P  L  I  S  C  V  G  A  I  H  C  N  I  L  A  I  R  T
 91  GGCAACGACTTTGCTGCCATTAAGCTACAGGTGATAAAATTGATCTATCTCATGATATGGCATTCGTTGGTGATTATCTCACCTGTAGTG
 31  G  N  D  F  A  A  I  K  L  Q  V  I  K  L  I  Y  L  M  I  W  H  S  L  V  I  I  S  P  V  V
181  ACTCTGGCATTCTTCCCTGCATCTCTGAAACAGGGGAGCTTACACTTTCTATTAATCATATATTTTGTATTATTGTTGACACCATGGCTG
 61  T  L  A  F  F  P  A  S  L  K  Q  G  S  L  H  F  L  L  I  I  Y  F  V  L  L  L  T  P  W  L
271  GAGTTTTCGAAAAGTGGAACTCATCTTCCTAGCAACACAAAAATAATTCCAGCATGGTGGGTAAGTATGGATGCTTATCTTAATCATGCT
 91  E  F  S  K  S  G  T  H  L  P  S  N  T  K  I  I  P  A  W  W  V  S  M  D  A  Y  L  N  H  A
361  AGTATATGCTGCCATCAATTCTCCTGCTTGTCAGCAGTGAAACTGCAGCTGTCAAATGAGGAATTGATAAGAGACACGAGGTGGGACATA
121  S  I  C  C  H  Q  F  S  C  L  S  A  V  K  L  Q  L  S  N  E  E  L  I  R  D  T  R  W  D  I
451  CAATCCTACACTACAGATTTCAGTTTTTAGaaaatgtgataatatattgatatttagtttcttggaggggaacgttttaccgaagtgtt
151  Q  S  Y  T  T  D  F  S  F  *   159
541  gtgactcaataattgccgtgtagttcatcaaaacctacatattagccctttggcttaagctccgcttctgtcagtatttgcaaccaaggt
631  ggtcgggcaaagtattgccaggagatactgaaaatcatccagaagcactgtgatattgtgtaagcatctggagaaaattcagttaaaaga
721  ataaaagtaagcagctgaggaattactatcactcatggagaagggtaggatattttcaataagtgagtatgcaatatccatatatacttt
811  cacagaacaaagagtaaagaggctgagtgtgtgacttttataaagatactcatgaaaaatataaacaacaaaaccttggaagtagtttctaat
901  aaaattgatttttctaaaaaaaaaa  925
```

FIG. 11

PTPRY

```
-182                                                                                          aa
-180 gaagaggagcacaccacaccagaaacagacatcttgcagtgtttcactgtctcaaccttatctgcacagtccgaggtcagtctgagagag
 -90 cttctgagagacccaggatgaagggatgcagtgaggtcaagagcccaaccttctttcactgacacccacctctaaggactcagaagagac

   1 ATGAATAAAATGGGCCTCAACAATCCCAAGAAGAACCACTCAAGGACAATGGGAGCCACTGGGCTTGGCTTCCTACTTCCCTGGAAACAA
   1 M  N  K  M  G  L  N  N  P  K  K  N  H  S  R  T  M  G  A  T  G  L  G  F  L  L  P  W  K  Q
  91 GACAATTTGAATGGCACTGACTGCCAGGGATGCAATATTTTATACTTCTCTGAGACTACGGGGAGCATGTGTTCTGAACTTTCCCTGAAC
  31 D  N  L  N  G  T  D  C  Q  G  C  N  I  L  Y  F  S  E  T  T  G  S  M  C  S  E  L  S  L  N
 181 AGAGGTCTTGAGGCCAGAAGGAAGAAGGATCTTAAAGACTCATTTCTCTGGAGATATGGGAAGGTTGGCTGTATCTCACTTCCACTTCGT
  61 R  G  L  E  A  R  R  K  K  D  L  K  D  S  F  L  W  R  Y  G  K  V  G  C  I  S  L  P  L  R
 271 GAGATGACCGCCTGGATTAACCCACCCCAAATTTCAGAGATTTTCCAAGGCTACCACCAGAGGGTGCACGGAGCTGATGCACTGAGCCTG
  91 E  M  T  A  W  I  N  P  P  Q  I  S  E  I  F  Q  G  Y  H  Q  R  V  H  G  A  D  A  L  S  L
 361 CAAACCAACTCTCTGAGAAGCAGGTTATCTTCACAGTGCCTCGGACAGAGCTTCCTTCTCAGGACACTCGAGAGAGCCGTGGTTTCAGGG
 121 Q  T  N  S  L  R  S  R  L  S  S  Q  C  L  G  Q  S  F  L  L  R  T  L  E  R  A  V  V  S  G
 451 CACTTGGGGACATCTGTGGCCACGTTCATGAAGAAGACTAAGCCTACTTCATCTCAGGACCCGCCCAAGAGTGGCCGCGGCTTTGGGACA
 151 H  L  G  T  S  V  A  T  F  M  K  K  T  K  P  T  S  S  Q  D  P  P  K  S  G  R  G  F  G  T
 541 CCTGCGGTCGGGTCCACCATGAGGATAAAACCTCCTTCTCTTCTGGACATGTCCAGGAGTGGCCGTTGCTACAAGTCACCTGGTGCTACG
 181 P  A  V  G  S  T  M  R  I  K  P  P  S  L  L  D  M  S  R  S  G  R  C  Y  K  S  P  G  A  T
 631 ACCAGGGTGAGAATAAAGACGTCTCCTCAGGACCCTCCCAGGAGAGTACATGGCATTGAGACATCTGGCGGCCAAGTGAGGAAAAGACAC
 211 T  R  V  R  I  K  T  S  P  Q  D  P  P  R  R  V  H  G  I  E  T  S  G  G  Q  V  R  K  R  H
 721 CCTGTCTGCAGCACCCAGAACTGAggaggggcactgccctgggccttacttcccagccctggcctccaattctgaccttacaaaagtgtc
 241 P  V  C  S  T  Q  N  *  247
 811 ccttgagtgaggcagtgaccacgcattgtcacagctaccaaagtgtggtttgcagatgatctgggcttgtttctggcagagattctggta
 901 cagagaaaggagaggcgttgagtggaaccacgatgggctgaggccaggggagacatcacaacctccaacaacactttttttcatgcttta
 991 ataaatcatttttcttagagaactaaagtagttgaaacaatatagaaacatttttaagtaggcataaaaaaaaaa   1066
```

FIG. 12

TTY1

tgtctgtcagagctgtcagcctgcttaagcagagtaaaatggtacaggcagtgcagcctggtagcgagaaaaaaggctgcctgtgaaatc
ccactgtgggaccataagtggggacctcagggccccttcatggcatctccatggccatgtcatgctggagaaggaggcgtttcaagaatg
tgagctgatcgctggaaactgctcatctgactccagtctcaaaagaggctatgtgcaagaatcgggtgaagttgtgagacccccatccacc
cctcacaagattgtatccccaccctgtctgaccttactgctgctcaaactatctgtccaaggatgaaaacccaggacaaaggaggagtaa
ccctcatgatgtgaagcacgtgttcacctgtgaatataacctgaggatcatgagactatctgtggatttcacagagaagacagacgagaa
gacaccgctgacacttctccacggaggtctccttttccaccaagatgcagatgcttcttgcaaggactatcctgtgaatcccacagagaa
gacaggtgtggttccaatgccggtgcacctccagggaattctccttctctaccaagctccaggccttctgccatgatcatgagactattt
gtggatttcacagagaagataggtgaaggtacagcatggcatccacccctcaccagaggggtatccccacccctatctgaccttattacc
ttattgctgttcaaagtctctatcccagactgaaatcccaagacaatggagaagttcccccctgatgatgtgaagcaccaactcctctggg
aatcaaattcgaggtaaatttaataggcccggtagagatgaatgatagtgtctctccttggattggctgaaagacaattaaacactggta
tatttctgttaaaaaaaaaa

FIG. 13

TTY2

aggcttgccatcaccacagatggcctctgagacactgtttgaaccacatctgcacctgtgagaggccagtttgaggtatgagaacactgt
ttcaatttggacttgcctttgtcttggttcctgctttcccagatggcacctacccaacccaggatgaatgagtgcagagaggtcaagtg
ccaggccatcttttgctgacacccttttctggtatttcaggtataagtccatcatccaaagactgctcaacatctcaccagaatatattt
caatcctcatggggcatgattctttcacaaaacccctttcaggaatggagtcagaagagtagtttccagagacaacctcacagtcttgga
acggctctgcctcccatgtgatctgaccatggagatggcatataagggccctaagtttgagactttagggtactgcaatgcgttatcac
aggcagcctttatcctgataccaagccagctctgcctgtaccatttcctctgcttaggcaggctgacagccctgacaccctggtgctcc
agtttgagtcactatatgtggatgtgctagtcttggggcaatggacctgagctgtgagctgtagctagtgtcacaatgaatgccagcttt
gctagtaacaattccctttggcttggtagagaaggggacctctgtggaggtacaatggtggtgcactgtcacctgtcttctctgtgggat
ccatgggacagttccatgatcctaggagagggtagatgtgagccagcctgaagaaatgtcaagcagagccccaggaatgaagcacaaaat
cactacagatccaaaaggatctgcagaatttgtcaggcctgcctagacattgtaggggttagtcttattgaaatgtgtcccactgtaatt
tccaacttcagccttcctgtgttcccagcagtttctctctcccaggtggggctttctgcagaatgacacagcctcagaagctactgggct
gtgtgttactgtgggagtgttgcgagtgttggatgtcagcatgtgtgtggctttgtgtgtgtgtaggtgtctgtgtgtgtgtatgt
aaatgaattctgtggatcaggaatcagcaatgactagttaagctgtctgtgaccagccgggttccccatcgtctgcccctgccaaaaaa
caggtactcttctacaaagaagaggagagcaccacacccaagaacagacatctcccagtgttgcattataaagcagccaacccacagaca
ctagcactctggtctgcatagcccctttaatttacctagaattcagttcccagccaagtaggtgcttcatgtcctgagggtgcaatcctc
catcatcttgagatttcatgctggtacagagagtgtgacagcaataaggtcagataggggtgagtatacaacctggtgaagggtggatgg
ggtcccgtaccttcaccagcaaaagggtgaaaatagatgacacagaatgtgcttccaactccatccccacattcccataattgcaaaat
cagtcaacaacatggcctggtgtttaggtgggagtactccaacctgcaggaagaatttggagtgcaaattgtggccaatctggaaaactc
ctggtttgagggttttaatacctgtagtcaaatggaagtggaatagattgatgctgggtgggttgtggcctccacatttgtgtcctcttt
tactgacttccattgtcctcattggtgtagggcttcctggatctggctcaacatcttccacactaaactcttccctgttcacagaagacc
atcataaaatgcattgtagaggccctgcaaggaccaggatgaagggagacagtgaggtcaagagcccagccatctttcactgacaccca
cttctgggttctcaggctggctgacaggtctgacagcccatcacgaaagcctgcatactcttagacacaaggactgagctatgggctcca
gctagcatcacaatgaaggccaccattgcctagggataagtccctgtgactttgtggataagaactccgtggagccaatccaaggagaga
cactatcattcatctctaccgggcctattaaatttacctcgaatttgattcccagaggagttggtgcttcacatcatcaggggaacttc
tccattgtcttgggatttcagtctgggatagagactttgaacagcaataagtttccttggtctggctcaacgtcttctaaactcaacatt
ccccagttcatggaaaatgatcctcatgggattctattgtaaaagctgcatgattcctggagaatgttttattgtcctggagtcaaccca
aaaatcaacactaccagtcatgttgcagagctccttgaattaaccttgaattcagtttccagctgagcagctgcttcacgttgtgagggg
gcaatcctccatcatcctgggatttcattctgggacacagagtttgagcagcaataaggctgggtccacattgcccctcaacagcattag
tggacatgattgtcagacttgcaatttccgcagacaccttctgtgaacattttcaacatcatctacatgagtgagagacccgttcgaca
tgtaagaatactgcttgactttggacctgcctttgtcgtggttcctgcctttctcatagatcccctgccaggcccaggatgataggaggc
aatgaagtcaagggccgagccccattcattgaaagctgactctggggtctcggtataattccatcacataaatcccctcaacaactca
ccagactatattccaatctccatggaacctgattcttgcacacagcctctttcaggaatggagtcagaagagcagtcttcagagaccacc
tcagtttggaaaagcctcctccttcagtggttcccagccatggagtcatcgtgaaggggctccatggtcaataatttacggtactgcac
ttggttatcacagacagactttttcatgatagcatgccatctctgtctatatcattttcctctgcttaggcaggctgacaactctgacag
ccaggggcccgaatctacctggcaaatgtgcatgctctactctcagtgcaaaaggcctgtttgggagttctgactagtgtcacaataaat
gccgccattgcctagtgaaaaaaaaaa

FIG. 14

Human *CDYL* (*CDY Like*)

ggagagaggacctatttctacctaaggacattcccggaaggcaatgggtttcaaacaatat
cctgaagagactcatctcggggaactaagcaggtggtaatcagagaacacagagcccccgg
aagaattttatggcatttcaggcaagccacaggccagcctggggaaaaagcaggaagaaaa
actggcaatacgagggcccaacccaaaagttattcctgaagagaaacaacgtgtcagcacc
agatgggccttcagaccccagcatctccgcgagcagtgagcaaagcggggcacagcagcct
cccggtttacaggttgaaaggattgttgacaaaaggaaaaataaaaaagggaagacagagt
atttggttcggtggaaaggctatgacagcgaggacgacacttgggagccggaacagcacct
cgtgaactgtgaggaatacatccacgacttcaacagacgccacacggagaagcagaaggag
agcacattgaccagaacaaacaggacctctcccaacaatgctaggaaacaaatctccagat
ccaccaacagcaacttttctaagacctctcctaaggcactcgtgattgggaaagaccacga
atccaaaaacagccagctgtttgctgccagccagaagttcaggaagaacacagctccatct
ctctccagccggaagaacatggacctagcgaagtcaggtatcaagatcctcgtgcctaaaa
gccccgttaagagcaggaccgcagtggacggctttcagagcgagagccctgagaaactgga
ccccgtcgagcagggtcaggaggacacagtggcacccgaagtggcagcggaaaagccggtc
ggagctttattgggccccggtgccgagagggccaggatggggagcaggcccaggatacacc
cactagtgcctcaggtgcccggccctgtgactgcagccatggccacaggcttagctgttaa
cgggaaaggtacatctccgttcatggatgcattaacagccaatgggacaaccaacatacag
acatctgttacaggagtgactgccagcaaaaggaaatttattgacgacagaagagaccagc
cttttgacaagcgattgcgtttcagcgtgaggcaaacagaaagtgcctacagatacagaga
tattgtggtcaggaagcaggatggcttcacccacatcttgttatccacaaagtcctcagag
aataactcactaaatccagaggtaatgagagaagtccagagtgctctgagcacggccgctg
ccgatgacagcaagctggtactgctcagcgccgttggcagcgtcttctgttgtggacttga
ctttatttattttatacgacgtctgacagatgacaggaaaagagaaagcactaaaatggca
gaagctatcagaaacttcgtgaatactttcattcaatttaagaagcccattattgtagcag
tcaatggcccagccattggtctaggagcatctatattgcctctttgcgatgtggtttgggc
taatgaaaaggcttggtttcaaacaccctataccaccttcggacagagtccagatggctgt
tctaccgttatgtttcccaagataatgggaggagcatctgcaaacgagatgctgctcagtg
gacggaagctgacagcgcaggaggcgtgtggcaagggcctggtctcccaggtgttttggcc
cgggacgttcactcaggaagtgatggttcgcattaaggagcttgcctcgtgcaatccagtt
gtgcttgaggaatccaaagccctcgtgcgctgcaacatgaagatggagctggagcaggcca
acgagagggagtgtgaggtgctgaagaaaatctggggctcggcccaggggatggactccat
gttaaagtacttgcagaggaagatcgatgagttctgagtgtcgggctgcccactggtgaca
ccgggatcgggctgagcaggagaacatcaccggctccagttcccctgatccattctcacag
cctgaaacaagctcacccgtagcttacgcttggaagcaggactgggaacatccacgctatt
tattatcgaggagttttaaagtactgtaactttaaaataaataactacaaagcttctttgt
cvaaacgtcattattttatacttatatacacgcaggtgtaaaagtataaaggtgagcacta
gactgctcttagaagctctaatttttgttttctttggctagtactgtataaaaaacagaat
tgtgttttattggttttggatgacagaaaagtctggaataatgtttgttttcctcatttct
tccttctagaacacagaatctaaggggtgttagccagcctcgcctccctgccccacgtag
agacacagagtgatgtgaggcgttggctttttctccaagaaggtacagatacctcagattc
gggaaactcaaaatcaaaagacttagcttctaggataaatacttctgatgaaaaatccgct
gaggagcataccccaaaccagacatatgcttaggattcatgctgagatatcaattggtttc
cccttctttttaaaatacgtccagttcttacccagttaacatgaagaaaccactgtctcta
gaagaaagcttgttttgcagtattagtgaatcactgaatagcttaagtatgactatctaag
ttataagttagtctttagtgggttttaaatagtttttctgacccttctgaaaaataactac
ataagtgcttcttgttgctgggtgagaaatactactttatagacagttttggttttctgtt
tgcagatatgattgatgtatttcaccaaaataaaatatttttatgtttataaagtgtaatt
tttaggttcacttagaatatatttttatttaataagttaaaattcttttggcacactattaa
atgcaaaaactcctttc

FIG. 15

Mouse *Cdyl* (*CDY like*)

ctttgaggtggtttagcatcccacttgttccttgaggacatctgttcctacctaagagcac
tcacctgagatgctcaaaggtccagaagaaacacttctcgggtgacaaagcaggtggtgac
cagagaacagaggccccccaaaaattttatggcattcaaggcaaagcacagccaacccgga
gggaaagcaagagtccagcctggaaatacatagcccaacccgaaggttatctctgaaggaa
aacaatgggcataggcaatagccagcctaattcacaggaagcccagctctgcacacttcca
gagaaagctgaacaacctactgatgataacacctgccagcaaaataatgtggttcctgcaa
cagtctcagaacccgatcaagcgtcccctgcaattcaagacgcggagactcaggtggaaag
tatcgttgacaaaaggaaaaacaagaaagggaagacagaatatctggtgcggtggaaaggc
tatgacagtgaggatgacacgtgggagcctgagcagcacctggtgaactgtgaggaataca
tccatgacttcaaccggcgccacaacgagaggcaaaaggaaggtagcctggctcgtgccag
cagagcctcccccagcaacgcccggaagcagatttccaggtccacccacagcactctctcc
aagaccaactccaaagcacttgtggtaggcaaagatcatgagtccaaaagcagccagctgt
tggctgccagccagaagttcaggaaaaacccagccccatctcttgcaaaccgcaagaacat
ggacctcgccaagtcagggatcaaaattctcgtgcctaagagccccgttaagggcaggacc
tcggttgatggctttcaggggagagccccgagaagctggaccctgtggatcagggtgccg
aggacactgtagccccagaggtgactgcagagaagcccactggggctttgctgggccctgg
tgcggagcgagccaggatggggagcaggccccgaatacatccactagtgcctcaggtttct
ggccccgtgactgctgccatggccacaggcttagctgttaatggaaaaggtacatctccat
tcatggatgcgctagcagccaacggaacagtcaccatacagacatccgtaacaggagtgac
agccgggaaaaggaaatttattgacgacagaagagaccaacctttgacaagcggttgcgt
ttcagtgtgaggcagacagagagtgcctacagatacagagatattgtcgtcaggaagcaag
atggcttcacccacatcttgttatccacaaaatcgtcagagaataactcactaaacccaga
ggtgatgaaagaagtrcagagcgccctgagcacagctgcagccgacgacagcaagctggtt
ctgctcagcgccgtgggcagcgtcttctgctgtggtctggactttatttatttttattcggc
gcctcacagatgaccgaaagagagaaagcactaaaatggcagacgctatcagaaacttcgt
gaatactttcattcagtttaagaagcctattattgtagctgttaatggcccagccattgga
ctaggagcatccatattgcctctttgtgatgtggtttgggctaacgaaaaggcttggtttc
aaacaccctataccaccttcggacagagtccagatggctgctctaccgttatgtttcccaa
gattatgggaggagcatctgcgaatgaaatgctgttcagtgggcggaagttgacggcacag
gaggcctgtggcaagggtctggtctcccaggtgttttggccaggaaccttcacacaggaag
tcatggttcgaatcaaggagctggcttcatgtaacccagttgtcctggaggaatccaaagc
cctggtgcgctgcaatatgaagatggagctagagcaggccaatgagagagaatgtgaagtg
ctgaagaagatctggggctccgcccagggcatggactccatgttaaagtacttacagagga
aaatcgatgagttctgatgggcaggctgagcaggacatcggtggctcccacttgctacgtc
gtcctgcagtggctcgtgcttggaggcagaactggaaacatccgagctatttattgccgcg
gagtttttaagtactgtaactttaaaataaatacaaagcttctttgtctaagcgtctttat
tttatactcatgtatacacaagtataaaaatgtaattgagcactaggctgctcttggaagc
tctaatttttcttgtaagctagttgtggatttttgttttgtttttgtttttaaaaggaatta
tgttttcatttttgggtgacagaagagtttgaaataatgtttgttttactctttttttttt
ccttaaatctagatcacagaccctcaaaattactagccagccttctccccctccctctact
gaaacatgtagaaatacttaaacatgttcctgcctctaggggggaggggggaggtgtgagta
cctcaatgctgaaaacagttctgatcaaacttaagaccaacctggtaaaaaaagcatcact
gatggaaaatcccacccacgggggcgtgggtttctgctgaaatgcccgccgctctaccttt
cttactgtcccattcttacccagccaccgtgaagagcccagtgtctggaggaaagcaggtg
gtccagtgtctgtgagtcactccgtagctcgagtgttacttgctaagttatgaattagcat
tagtgggtttaaatagtttttctgacccttttgaaaaataactacataagtactccttgt
ggctgggtgagaaatactactttgcatagttttgtttgtctatctgcagatatgattgctg
tattacaccaaaagtatttttttatgtttataaagtgtaatttttaggttcacttagaatat
attttatttaatttaaaattctcttggcacactattaaatacgtaaactcctttc

FIG. 16

Human *VCP* (*Variably Charged Protein*) family

VCP2r (VCP with 2 repeats)

gttgcgagacgttgagctgcggaagatgagtccaaagccgagagcctcgggacctccggcc
aaggccacggaggcaggaaagaggaagtcctcctctcagccgagccccagtgacccgaaga
agaagactaccaaggtggccgagaagggaaaagcagttcgtagagggagacgcgggaagaa
aggggctgcgacaaagatggcggccgtgacggcacctgaggcggagagcgggccagcggca
cccggccccagcgaccagcccagccaggagctccctcagcacgagctgccgccggaggagc
cagtgagcgaggggacccagcacgacccccgagtcaggaggccgagctggaggaaccact
gagtcaggagagcgaggtggaagaaccactgactgtgtggatggccagcttttcccctgtc
tccgagagcagcgactaagttcaggcccagccgccagacctcagagatctcaccagcgggg
tgcttgccattctgaagataataaaatgaatgtgttgcaaattgaaaaaaaaaa

FIG. 17A

VCP8r (VCP with 8 repeats)

cggaagatgagtccaaagccgagagcctcgggacctccggccaaggccacggaggcaggaa
agaggaagtcctcctctcagccgagccccagtgacccgaagaagaagactaccaaggtggc
caagaagggaaaagcagttcgtagagggagacgcgggaagaaaggggctgcgacaaagatg
gcggccgtgacggcacctgaggcggagagcgggccagcggcacccggccccagcgaccagc
ccagccaggagctccctcagcacgagctgccgccggaggagccagtgagcgaggggaccca
gcacgacccccctgagtcaggaggccgagctggaggaaccactgagtcaggagagcgaggtg
gaagaaccactgagtcaggagagccaggtggaggaaccactgagtcaggagagcgaggtgg
aggaaccgctgagtcaggagagccaggtggaagaaccactgagtcaggagagcgaggtgga
ggaaccactgagtcaggagagccaggtggaggaaccactgagtcaggagagcgagatggaa
gaactaccgagtgtgtagacggccagctactcccctatctccgagagcagcgactaagttc
aggcccagccgccagacctcagagatctcaccagcggggtgcttgccattctgaagataat
aaaatgaatgtgttgcaaattgaaaaaaaaaa

FIG. 17B

VCP10r (VCP with 10 repeats)

cgttgcgagacgttgagctgcggaagatgagtccaaagccgagagcctcgggacctccggc
caaggccacggaggcaggaaagaggaagtcctcctctcagccgagccccagtgacccgaag
aagaagactaccaaggtggccaagaagggaaaagcagttcgtagagggagacgcgggaaga
aaggggctgcgacaaagatggcggccgtgacggcacctgaggcggagagcgggccagcggc
acccggccccagcgaccagcccagccaggagctccctcagcacgagctgccgccggaggag
ccagtgagcgaggggacccagcacgacccccctgagtcaggaggccgagctggaggaaccac
tgagtcaggagagcgaggtggaagaaccactgagtcaggagagccaggtggaggaaccact
gagtcaggagagcgaggtggaagaaccactgagtcaggagagccaggtggaggaaccactg
agtcaggagagcgaggtggaggaaccactgagtcaggagagccaggtggaggaaccactga
gtcaggagagcgagatggaagaaccactgagtcaggagagccaggtggaggaaccaccgag
tcaggagagcgagatggaagaactaccgagtgtgtagacggccaagtactcccctatctcc
gagagcagcgactaagttcaggcccagccgccagacctcagagatctcaccagcggggtgc
ttgccattctgaagataataaaatgaatgtgttgcaaattgaaaaaaaaaa

FIG. 17C

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(72) Inventors; and
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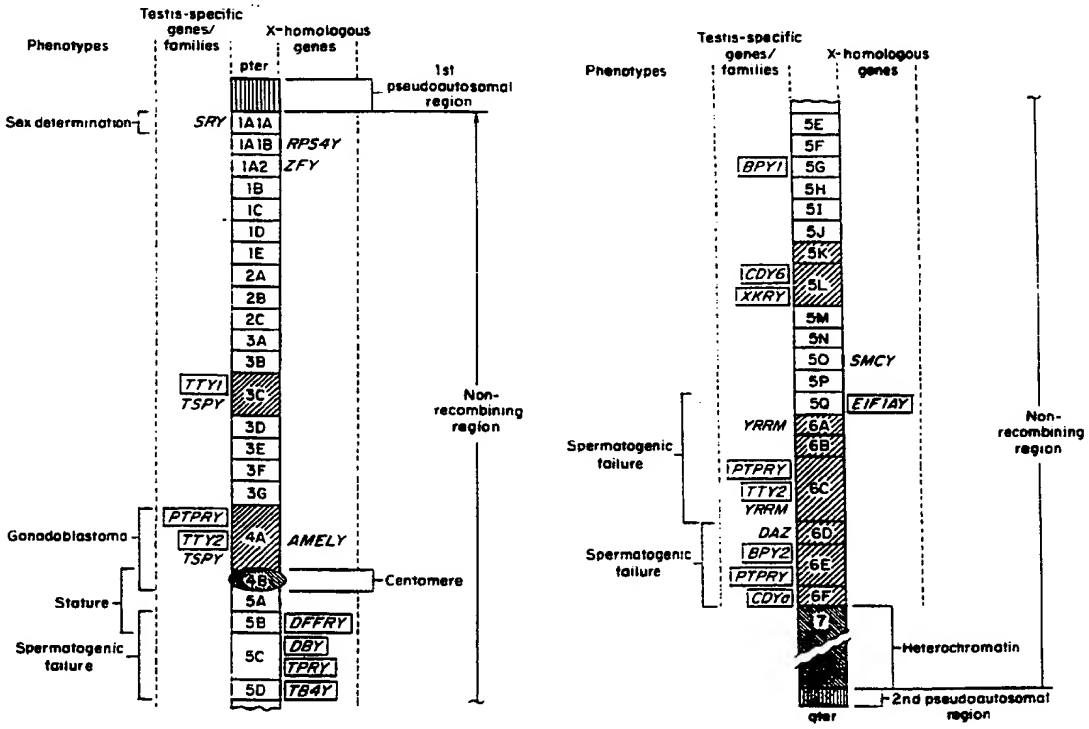

(57) Abstract

Genes of the non-recombining region of the human Y chromosome, which fall into two classes: X-homologous DNA which is expressed in many organs and has functional X homologs and testis-specific DNA.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07115

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 00375 A (IMP CANCER RES TECH) 9 January 1992 see the whole document ---	1
X	ZHANG J. ET AL.: "Molecular isolation and characterization of an expressed gene from the human Y chromosome" HUMAN MOLECULAR GENETICS, vol. 1, no. 9, December 1992, pages 717-726, XP002080218 see the whole document --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
12 October 1998	17. 12. 98

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INTERNATIONAL SEARCH REPORT

Interna[illegible]nal Application No
PCT/US 98/07115

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MA K. ET AL.: "A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis" CELL, vol. 75, no. 7, 31 December 1993, pages 1287-1295, XP002017338 cited in the application see the whole document	1,2
X	WO 95 11300 A (MEDICAL RES COUNCIL ;CHANDLEY ANN CHESTER (GB); KUN MA (GB); SHARK) 27 April 1995 see the whole document	1,2
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A	WO 96 41007 A (PROMEGA CORP) 19 December 1996 see the whole document	1-4,8
A	FOOTE S. ET AL.: "The human Y chromosome: overlapping DNA clones spanning the euchromatic region" SCIENCE, vol. 258, 2 October 1992, pages 60-66, XP002080220 see the whole document	1-4,8
P,X	LAHN B. AND PAGE D.: "Functional coherence of the human Y chromosome" SCIENCE, vol. 278, 24 October 1997, pages 675-680, XP002080221 see the whole document	1-4,8

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/07115

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4,8 partially (subject 1. on continutation-sheet)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-4,8 partially

   Isolated DNA which occurs on the non-recombining region of the human Y chromosome or the complement thereof, being testis-specific and optionally occuring in multiple copies on the Y chromosome.
   Said DNA being the CDY gene, a characteristic portion, a probe or complement thereof, a DNA which hybridizes thereto under stringent conditions.
   Said DNA having the SEQ ID NO:37,38 and coding for the amino acid of SEQ ID NO:39,40.

2. Claims: 1-4,8 partially

   idem for BPY 1, SEQ ID NO:41,42

3. Claims: 1-4,8 partially

   idem for BPY 2, SEQ ID NO:43,44

4. Claims: 1-4,8 partially

   idem for XKRY, SEQ ID NO:45,46

5. Claims: 1-4,8 partially

   idem for PTPRY, SEQ ID NO:47,48

6. Claims: 1-4,8 partially

   idem for TTY 1, SEQ ID NO:49

7. Claims: 1-4,8 partially

   idem for TTY 2, SEQ ID NO:50

8. Claims: 5-7,9 partially

   Isolated DNA which occurs on the non-recombining region of the human Y chromosome or the complement thereof, not being testis-specific and having a homolog on the human X chromosome.
   Said DNA being the DBY gene; a characteristic portion, a probe or complement thereof, a DNA which hybridizes thereto under stringent conditions.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Said DNA having the SEQ ID NO:17 and coding for the amino acid of SEQ ID NO:18.

9. Claims: 5-7,9 partially

idem for TPRY, SEQ ID NO:19,20,21,22,23,24

10. Claims: 5-7,9 partially

idem for TB4Y, SEQ ID NO:26,28

11. Claims: 5-7,9 partially

idem for EIF1AY, SEQ ID NO:30,32

12. Claims: 5-7,9 partially

idem for DFFRY, SEQ ID NO:34,36

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07115

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		EP 0832288 A	01-04-98
		US 5840549 A	24-11-98

CORRECTED VERSION*

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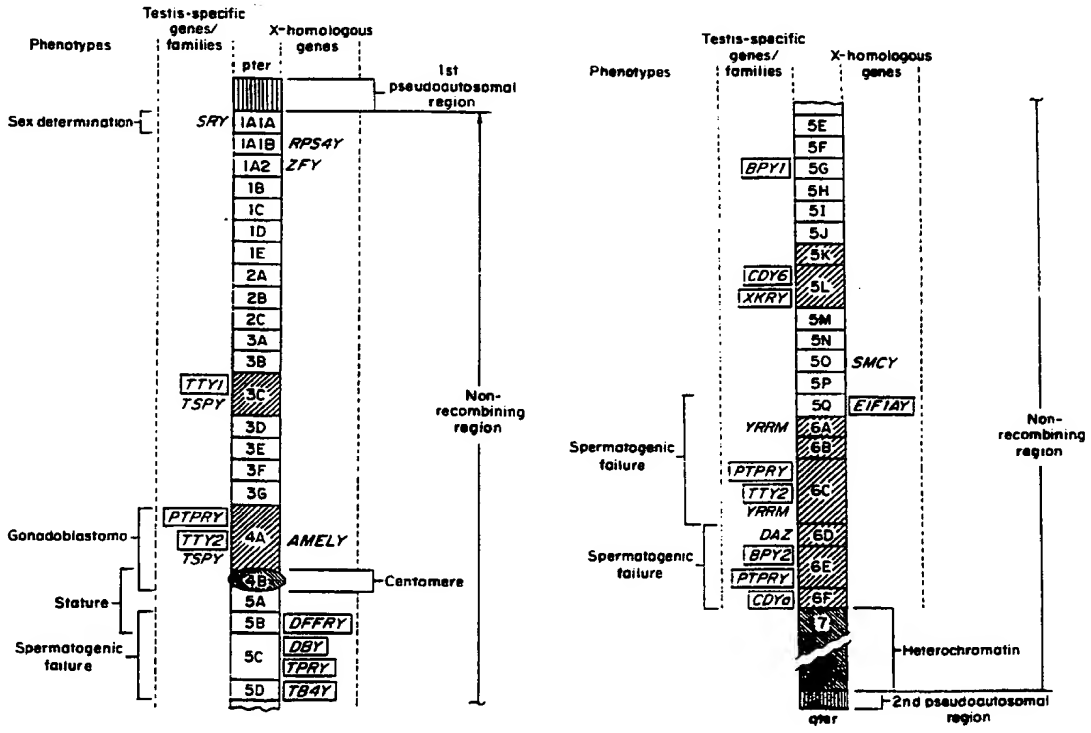

(57) Abstract

Genes of the non-recombining region of the human Y chromosome, which fall into two classes: X-homologous DNA which is expressed in many organs and has functional X homologs and testis-specific DNA.

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GENES IN THE NON-RECOMBINING REGION OF THE Y CHROMOSOME

GOVERNMENT SUPPORT

The invention described herein was made in whole or in part with government support under Grant Number HG00257 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/041,877, filed April 11, 1997, entitled "Genes in the Non-Recombining Region of the Y Chromosome" by Bruce T. Lahn and David C. Page. The entire teachings of the above referenced application is expressly incorporated herein by reference.

BACKGROUND OF THE INVENTION

The human Y chromosome is distinguished from all other nuclear chromosomes by four characteristics: the absence of recombination, its presence in males only, its common ancestry and persistent meiotic relationship with the X chromosome, and the tendency of its genes to degenerate during evolution (J. J. Bull, *Evolution of Sex Determining Mechanisms* (Benjamin Cummings, Menlo Park, CA, 1983); J. A. Graves, *Annu. Rev. Genet. 30*:233 (1996); B. Charlesworth, *Curr. Biol. 6*:149 (1996); W. R. Rice, *BioScience, 46,* 331

(1996)). To be precise, these distinctive characteristics apply only to the non-recombining portion or region of the Y chromosome (NRY), which comprises 95% of the human Y chromosome. The remaining 5% of the chromosome is composed of two pseudoautosomal regions that maintain sequence identity with the X chromosome by meiotic recombination (H. J. Cooke *et al.*, *Nature 317*:687 (1985); M. C. Simmler *et al.*, *Nature 317*:692 (1985); D. Freije *et al.*, *Science 258*:1784 (1992); G. A. Rappold, *Hum. Genet. 92*:315 (1993)). Given the NRY's peculiar characteristics, one might expect its gene content to be idiosyncratic. Since discovery of the Y chromosome in 1923, its gene content has been the subject of speculation. By the middle of this century, while studies of human pedigrees had identified many traits exhibiting autosomal or X-linked inheritance, no convincing cases of Y-linked inheritance could be found (T. S. Painter, *J. Exp. Zool.* (1923); C. Stern, *Am. J. Hum. Genet. 9*:147 (1957)). As a result, consensus began to emerge that the Y chromosome carried few, if any, genes. In 1959, reports of XO females and XXY males established the existence of a sex-determining gene on the human Y chromosome (P. A. Jacobs *et al. Nature 183*:302 (1959); C. E. Ford *et al.*, *Lancet, i*:711 (1959)), but this was perceived as a special case on a generally desolate chromosome. Opinions began to change only during the past decade, when eight NRY transcription units (or families of closely related transcription units) were identified, most during regionally focused, positional cloning experiments (D. C. Page *et al.*, *Cell 51*:1091 (1987); A. H. Sinclair *et al.*, *Nature 346*:240-244 (1990); J. Arnemann *et al.*, *Genomics 11*: 108 (1991); E. C. Salido *et al.*, *Am. J. Hum. Genet. 50*:303 (1992); E. M. Fisher *et al.*, *Cell 63*:1205 (1990); K. Ma *et al.*, *Cell 75*:1287 (1993); A. I. Agulnik *et al.*, *Hum. Mol. Genet. 3*:879 (1994); R. Reijo *et al.*, *Nat.*

Genet. 10:383 (1995)). It was not known if there were more genes in the NRY.

SUMMARY OF THE INVENTION

A systematic search of the non-recombining region of the human Y chromosome (NRY) has identified 12 novel genes or gene families. All 12 novel genes, and six of eight NRY genes or families previously isolated by less systematic means, fall into two classes. The first class of genes exists in one copy and is expressed in many organs; they have functional X homologs that escape X inactivation, as predicted for genes involved in Turner (XO) syndrome. The second class consists of Y-chromosomal gene families expressed specifically in testes, and may account for infertility among men with Y deletions.

The genes described herein, portions of the genes and DNA which hybridizes to genes or gene portions described are useful in diagnostic methods, such as a method to identify individuals in whom all or a portion of a gene or genes of the NRY is missing or altered. For example, Y chromosomal DNA from males with a known condition, such as infertility or reduced sperm count, can be assessed, using the gene(s) described herein, or characteristic portions thereof, to determine whether their DNA lacks some or all of the gene(s) described herein or contains an altered gene(s) (e.g., a gene in which there is a deletion, substitution, addition or mutation, compared to the sequences presented herein). Y chromosomal DNA (e.g., from a male with reduced sperm count or viability) can be assessed, using DNA described herein or DNA which hybridizes to DNA described herein, to determine whether the condition is associated with or caused by the occurrence of the gene or the gene alteration. For example, the presence or absence of all or a portion of a gene or genes shown to be necessary for fertility or

adequate sperm count can be assessed, using DNA which hybridizes to the gene or genes of interest to determine the basis for their infertility or reduced sperm count. In one embodiment, the occurrence of one or more Y-specific genes or a characteristic portion of one or more Y-specific genes is assessed in Y chromosomal DNA. In another embodiment, deletion or alteration of one of the testis-specific (Y-specific) genes described is assessed, such as by a hybridization method in which DNA which hybridizes to one of the Y-specific genes described herein or a characteristic portion thereof is used to assess a DNA sample obtained from a male who has a reduced sperm count. Lack of hybridization of the Y-specific DNA used to DNA in the sample indicates that the gene is not present in sample DNA or is present in an altered form which does not hybridize to Y-specific DNA of the present invention. In another embodiment, an X-homologous gene or genes present on the NRY can be used to determine whether the gene is present in an individual or if it occurs in an altered form in the individual. Using known methods, such as hybridization methods, X or Y chromosomal DNA from an individual can be assessed for the presence or absence of one or more of the X-homologous genes or a characteristic portion of one or more X-homologous genes. X or Y chromosomal DNA can also be assessed for the presence or absence of an altered form of one or more of the X-homologous genes described. In the present methods, DNA can be analyzed for the occurrence of Y-specific DNA, X-homologous genes or both. For example, a "battery" or group of DNA probes (sequences) can be used to analyze sample DNA; the probes can include Y-specific DNA probes (e.g., DNA which hybridizes to a Y-specific gene), X-homologous gene probes (e.g., DNA which hybridizes to an X-homologous gene) or both types of probes. DNA described herein is also useful as primers in an amplification

method, such as PCR, useful for identifying and amplifying Y-specific DNA or X-homologous genes in a sample (e.g., Y chromosomal DNA). Further, proteins or peptides encoded by the DNA described herein, such as proteins or peptides encoded by an X-homologous gene or proteins or peptides encoded by testis-specific DNA (a testis-specific gene), can be assessed in samples. This can be carried out, for example, using antibodies which recognize proteins or peptides of the present invention (proteins or peptides encoded by DNA described herein).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a gene map of the non-recombining region of the Y chromosome.

Figure 2 shows the amino acid sequence alignments of the chromodomain (SEQ ID NO.: 1-6) and putative catalytic domain (SEQ ID NO.: 7-12) of human CDY genes with their respective homologs. Amino acid identities are indicated by black shading and for each protein, the first and last amino acid residues are numbered (with respect to the initiator methionine) and the total length of the protein is indicated. Chromodomain: SEQ ID NO.: 1, CDY (human); SEQ ID NO.: 2, HP1 (Drosophila); SEQ ID NO.: 3, Polycomb (Drosophila); SEQ ID NO.: 4, CHD1 (Drosophila); SEQ ID NO.: 5, Su(var) 3-9 (Drosophila; SEQ ID NO.: 6, PDD1 (Tetrahymena); SEQ ID NO.: 7; Covalent modification domain: SEQ ID NO.: 8, CDY (human); SEQ ID NO.: 9, Enoyl-CoA Hydratase (Human); SEQ ID NO.: 10, 4-CBA-CoA dehalogenase (Arthrobacter); SEQ ID NO.: 11, Crotonase (C. acetobutylicum); SEQ ID NO.: 12, Naphthoate synthase (E. coli).

Figures 3A and 3B are the nucleic acid sequence of DBX (long and short transcripts, SEQ ID NO: 13 and SEQ ID NO: 14, respectively) and the encoded amino acid sequences (SEQ ID NO: 15 and SEQ ID NO.: 16, respectively), DBY (SEQ ID

NO: 17) and the encoded amino acid sequence (SEQ ID NO: 18). Dots in the DBX DNA and protein sequences indicate that the nucleic acids or amino acid residues are the same as those represented for DBY; dashes indicate a missing nucleic acid or amino acid residue.

Figures 4A and 4B present the nucleic acid sequences for three forms of TPRY (short, medium and long, SEQ ID NO: 19, SEQ ID NO: 20 and SEQ ID NO: 21, respectively) and the encoded amino acid sequences for the short, medium and long forms (SEQ ID NO: 22, SEQ ID NO.: 23 and SEQ ID NO: 24, respectively).

Figure 5 presents the nucleic acid sequences of TB4X (SEQ ID NO: 25) and TB4Y (SEQ ID NO: 26) and the encoded amino acid sequences (SEQ ID NO: 27 and SEQ ID NO: 28, respectively). Dots in the TB4X DNA and protein sequences indicate that the nucleic acids or amino acid residues are the same as those represented for TB4Y.

Figure 6 represents the nucleic acid sequences of EIF1AX (SEQ ID NO: 29) and EIF1AY (SEQ ID NO: 30) and the encoded amino acid sequences (SEQ ID NO: 31 and SEQ ID NO: 32, respectively).

Figures 7A - 7D represent the nucleic acid sequences of DFFRX (SEQ ID NO: 33) and DFFRY (SEQ ID NO: 34) and the encoded amino acid sequences (SEQ ID NO: 35 and SEQ ID NO: 36, respectively).

Figure 8 represents the nucleic acid sequences of CDYa (SEQ ID NO: 37) and CDYb (SEQ ID NO: 38) and the encoded amino acid sequences (SEQ ID NO: 39 and SEQ ID NO: 40, respectively).

Figure 9 represents the nucleic acid sequences of BPY1 (SEQ ID NO: 41) and the encoded amino acid sequence (SEQ ID NO: 42).

Figure 10 represents the nucleic acid sequence of BPY2 (SEQ ID NO: 43) and the encoded amino acid sequence (SEQ ID NO: 44).

Figure 11 represents the nucleic acid sequences of XKRY (SEQ ID NO: 45) and the encoded amino acid sequence (SEQ ID NO: 46).

Figure 12 represents the nucleic acid sequences of PTPRY (SEQ ID NO: 47) and the encoded amino acid sequence (SEQ ID NO: 48).

Figure 13 is the nucleic acid sequence of TTY1 (SEQ ID NO: 49).

Figure 14 is the nucleic acid sequence of TTY2 (SEQ ID NO: 50).

Figure 15 shows the nucleic acid sequence of the human CDY Like (CDYL) gene, which is the human autosomal homolog of CDY, located on chromosome 6p and expressed ubiquitously.

Figure 16 shows the nucleic acid sequence of the mouse Cdyl (CDY like) gene, which is the mouse ortholog of human CDYL, located on chromosome 13 and expressed predominantly in the testis. A longer transcript of the gene is ubiquitously expressed.

Figures 17A - 17C show the nucleic acid sequences of human Variably Charged Protein family members VCP2r, VCP8r and VCP10r, which are expressed in the testis and highly polymorphic.

Figure 17A is the nucleic acid sequence of VCP2r.

Figure 17B is the nucleic acid sequence of VCP8r.

Figure 17C is the nucleic acid sequence of VCP10r.

DETAILED DESCRIPTION OF THE INVENTION

Y chromosome genes, classed as genes having X homologues and testis-specific (Y-specific) genes, are the subject of the invention described herein, as are DNA which hybridize to (are complementary to) all or characteristic portions of the Y chromosome genes, the encoded products (e.g., proteins, peptides, glycoproteins), antibodies and methods of diagnosis or treatment in which the genes,

complementary DNA, encoded proteins or antibodies are used. As described herein, fragments that hybridized to Y chromosomal DNA were selected and then their nucleotide sequences determined. It was expected that these sequence fragments would represent a redundant sampling of a much smaller set of genes. Computer analysis revealed that 577 fragments corresponded to known Y genes, including seven of eight NRY genes and all eight pseudoautosomal genes previously reported. These findings suggested that the 2539 sequence fragments represented the great majority of all Y-chromosomal genes. After further analysis, both to eliminate human repetitive sequences and to assemble overlapping fragments into contigs, 912 novel and non-overlapping sequences were hybridized to Southern blots of human genomic DNAs. 308 sequences that detected at least one prominent male-specific fragment were judged likely to derive from the NRY, and for each work was carried out to isolate cDNA clones from a human testis library, as described in Example 1. Nucleotide sequencing of cDNA clones, and rescreening of libraries as necessary, yielded full-length cDNA sequences for ten novel NRY genes or families, and partial cDNA sequences for two additional ones (Table and Figures 1 - 14).

TABLE: 12 Novel Genes or Families in the NRY

Gene Symbol	Gene Name	Tissue Expression	Multi-copy on Y	X homolog	Escape x Inactivation
DBY	Dead Box Y	ubiquitous		DBX	yes
TB4Y	Thymosin β4, Y isoform	ubiquitous		TB4X	yes
EIF1AY	Translation Initiation Factor 1A, Y isoform	ubiquitous		EIF1AX	yes
TPRY	TPR motif Y	ubiquitous		TPRX	yes
DFFRY	Drosophila Fat Facets Related Y	ubiquitous		DFFRX	yes
CDY	Chromodomain Y	testis	yes		
BPY1	Basic Protein Y 1	testis	yes		
BPY2	Basic Protein Y 2	testis	yes		
XKRY	XK Related Y	testis	yes		
PTPRY	Protein-Tyrosine Phosphatase Related Y	testis	yes		
TTY1	Testis Transcript Y 1	testis	yes		
TTY2	Testis Transcript Y 2	testis	yes		

All 12 novel genes were localized on the Y chromosome, as described in Example 2. Figure 1 is a gene map of NRY. As shown, the Y chromosome consists of a large non-recombining region (NRY; euchromatin plus heterochromatin) flanked by pseudoautosomal regions (pter, short arm telomere; qter, long arm telomere). The NRY is divided into 43 ordered intervals (1A1A through 7) which are defined by naturally occurring deletions (D. Vollrath, *et al.*, *Science 258:*52 (1992)). Listed immediately above the Y chromosome in Figure 1 are nine NRY genes with functional X homologs; novel genes are boxed. Indicated immediately below the Y chromosome are 11 testis-specific genes or families, some with multiple locations. It is likely that some testis-specific families have members in additional deletion intervals; the locations indicated are representative, but are not necessarily exhaustive. At the bottom of Figure 1 are shown NRY regions implicated, by deletion mapping, in sex determination, germ cell tumorigenesis (gonadoblastoma), stature, and spermatogenic failure (K. Ma *et al.*, *Cell 75:*1287 (1993); R. Reijo *et al.*, *Nat. Genet. 10:*383 (1995); P. H. Vogt *et al.*, *Hum. Mol. Genet. 5:*933 (1996); J. L. Pryor *et al.*, *New England J. Med. 336:*534 (1997); K. Tsuchiya *et al.*, *Am. J. Hum. Genet. 57:*1400 (1995); P. Salo *et al.*, *Hum. Genet. 95:*283 (1995)). Euchromatic regions that are made up, at least partially, of Y-specific repeats are drawn in grey. *AMELY*, which appears to fall within such a repeat-containing region, is actually located in a sub-region of 4A that is not repetitive.

Expression of the 12 novel genes was assessed in diverse human tissues, by Northern blotting. Autoradiograms were produced by hybridizing ^{32}P-labeled cDNA probes to Northern blots of poly(A)$^+$ RNAs (2 μg/lane) from human tissues (Clontech, Palo Alto, CA). Probes employed were cDNA clones, full-length (most genes) or

partial (*DBY*, nucleotides 1476-2319 of GenBank AF000985; *TPRY*, nucleotides 861-1768 of GenBank AF000996; *DFFRY*, nucleotides 8604-9878 of GenBank AF000986). Blots were hybridized at 65°C in Church's buffer (0.5 M Na_1PO_4 at pH7.5, with 7% SDS), and washed at 65°C in 1X SSC and 0.1% SDS. *DBY*, *TB4Y*, *EIF1AY* and *DFFRY* probes cross-hybridize to transcripts derived from their X homologs. For all five X-homologous genes (*DBY*, *TPRY*, *TB4Y*, *EIF1AY* and *DFFRY*), expression was tested and confirmed in three male tissues (brain, prostate and testis) by RT-PCR using Y-specific primers.

The novel genes encode an assortment of proteins and are dispersed throughout the euchromatic portions of the NRY. Nonetheless, all 12 genes fall into two discrete classes: 1) X-homologous genes and 2) testis-specific, Y-specific gene families (Table).

The X-homologous genes share the following characteristics: each has a homolog on the X chromosome encoding an extremely similar but nonidentical protein isoform, each is expressed in a wide range of human tissues (is not testis-specific), and each appears to exist in a single copy on the NRY. There are five novel representatives of this X-homologous class:

1. *DBY* encodes a novel "DEAD box" protein, perhaps an RNA helicase involved in translation initiation (P. Linder, et al., *Nature, 337*, 121 (1989); R.-Y. Chuang, P. L. Weaver, Z. Liu, T.-H. Chang, *Science, 275*, 1468 (1997)). The DBY protein is 91% identical to DBX, encoded by a homologous gene on the human X chromosome.
2. *TPRY* encodes a novel protein containing 10 tandem "TPR" motifs, a protein-protein interaction domain found in the products of the yeast SSN6/CYC8, CDC16, and CDC23 genes, among others (R. S. Sikorski, M. S. Boguski, M. Goebl, P. Hieter, *Cell, 60*, 307 (1990); D. Tzamarias, K. Struhl, *Genes Dev, 9*, 821 (1995)). Differential splicing may

generate TPRY isoforms that differ at their carboxy termini. The amino terminal portion of the TPRY protein is 83% identical to TPRX, encoded by an homologous gene on the X chromosome.

3. *TB4Y* encodes a 44 amino acid protein that differs at only three residues from thymosin ß$_4$, which functions in actin sequestration (H. Gondo, et al., *J. Immunol. 139:*3840 (1987); D. Safer, M. Elzinga, V. T. Nachmias, *J Biol Chem, 266,* 4029 (1991)), and we found is located on the X. It is proposed that the X-linked gene encoding thymosin ß$_4$ be called *TB4X*.

4. *EIF1AY* encodes a Y-linked isoform of translation initiation factor 1A (eIF-1A) (T. E. Dever, et al., *J Biol Chem, 269,* 3212 (1994); J. W. Hershey, *Annu. Rev. Biochem. 60,* 717 (1991)), which we discovered is located on the X. It is proposed that the X-linked gene encoding eIF-1A be called *EIF1AX*. The amino acid sequences of the X and Y-encoded proteins are 97% identical.

5. *DFFRY* encodes a Y-linked isoform of DFFRX, a recently described X-linked protein. A Y-linked homolog was detected previously, but had been thought to be a pseudogene. The human DFFRX and DFFRY proteins, which are 91% identical, are homologous to the Drosophila *fat-facets* gene product, a deubiquinating enzyme required for eye development and oogenesis (M. H. Jones, et al., *Hum Mol Genet* 5, 1695 (1996); J. A. Fischer-Vize, G. M. Rubin, R. Lehmann, *Development, 116,* 985 (1992); Y. Huang, R. T. Baker, J. A. Fischer-Vize, *Science, 270,* 1828 (1995)).

The second group of novel NRY genes, the testis-specific, Y-specific gene families, share a very different set of characteristics: each appears to be expressed specifically in testes and each appears to exist in multiple copies on the NRY, as judged by I) the number and intensity of hybridizing fragments on genomic Southern blots or ii) multiple map locations on the Y. We report

five novel testis-specific, Y-specific gene families with full-length cDNA sequences:

1. The *CDY* family encodes proteins with an amino-terminal "chromodomain," a chromatin binding motif (T. C. James, S. C. Elgin, *Mol Cell Biol, 6,* 3862 (1986); B. Tschiersch, et al., *EMBO J, 13,* 3822 (1994); R. Paro, D. S. Hogness, *Proc Natl Acad Sci U S A, 88,* 263 (1991); D. G. Stokes, K. D. Tartof, R. P. Perry, *Proc Natl Acad Sci U S A, 93,* 7137 (1996); M. T. Madireddi, et al., *Cell, 87,* 75 (1996)) (Figure 3). The carboxy-terminal half shows striking amino acid similarity, over a region of more than 200 residues, to nearly the full length of several enzymes, both prokaryotic and eukaryotic (M. Kanazawa, et al., *Enzyme Protein, 47,* 9 (1993); A. Schmitz, K. H. Gartemann, J. Fiedler, E. Grund, R. Eichenlaub, *Appl. Environ. Microbiol. 258,* 4068 (1992); Z. L. Boynton, G. N. Bennet, F. B. Rudolph, *J Bacteriol, 178,* 3015 (1996); V. Sharma, K. Suvarna, R. Meganathan, M. E. Hudspeth, *J Bacteriol, 174,* 5057 (1992); P. M. Palosaari, et al., *J Biol Chem, 266,* 10750 (1991)). The reactions catalyzed by these homologs are diverse, but in each case the substrate contains cofactor A (CoA) attached to a carbonyl group, and an alkoxide intermediate is formed. The unprecedented combination of a chromodomain and a putative CoA-substrate enzyme in a single polypeptide suggests that, in vivo, CDY proteins may catalyze covalent modification of DNA or chromosomal proteins, perhaps during spermatogenesis.

2. The *BPY1* genes encode a basic protein, 125 residues long, with little sequence similarity to known proteins. The encoded protein is rich in serine, lysine, arginine, and proline and has a pI of 9.4. Southern blotting studies revealed homologous sequences on the human X chromosome, but screening of cDNA libraries has failed to yield X-derived clones.

3. The *BPY2* genes encode a second basic protein, 106 residues in length, without obvious sequence similarity to BPY1 or other known proteins. The pI of BPY2 is 10.0.

4. The *XKRY* genes encode a protein with sequence similarity to XK, a putative membrane transport protein defective in McLeod syndrome (M. Ho, et al., *Cell*, 77, 869 (1994)).

5. The *PTPRY* genes encode a protein with weak homology to a putative protein-tyrosine phosphatase (PTPase) in the mouse (W. Hendriks, et al., *J Cell Biochem*, *59*, 418 (1995)). Two additional families of testis-specific transcription units, referred to as *TTY1* and *TTY2*, have been identified. The sequences represented in Figures 14 and 15 are being assessed for open reading frames.

It appears that conventional single-copy genes, commonplace elsewhere in the genome, are quite uncommon in the NRY. Indeed, the two classes of NRY genes suggested by the systematic search described herein accommodate not only the 12 genes reported here, but also six of eight previously identified NRY genes. *SRY*, a Y-specific gene that triggers the male pathway of sexual differentiation, is expressed in testes, and exists in only one copy in the NRY. *AMELY*, which has an X-linked homolog *AMELX*, is expressed only in the developing tooth bud. The X inactivation status of *AMELX* is unknown.

Also described herein are five additional genes and their sequences (Figures 15, 16, 17A - 17C): human CDY Like (CDYL), which is the human homolog of CDY; it is on chromosome 6p and expressed ubiquitously; mouse Cdyl (CDY like), which is the mouse ortholog of human CDYL; it is on chromosome 13 and expressed predominantly in testis and also has a longer transcript that is expressed ubiquitously; and human VCP (Variably Charged Protein) family, which is a family of genes on the X chromosome that are homologous to BPYI, expressed in the testis and highly

polymorphic. Human CDY, human CDYL and mouse Cdyl have been shown to be histone acetyltransferases by *in vitro* assays. Human CDY is a candidate for the Azoospermia Factor (AZF) because it is within the AZFc region that is commonly deleted in infertile men. Chemicals that block the enzymatic activity of any of these genes are candidate male contraceptives.

Inhibitors of the enzymatic activity of these genes, such as the human CDY gene, can be identified through an *in vitro* assay. For example, the protein encoded by one of the genes (e.g., CDY-encoded protein) can be produced, such as by recombinant means (e.g., in bacterial cells containing a vector or plasmid which includes the gene to be expressed), and obtained. The effect of a candidate inibitor (drug) on the enzymatic activity of the protein can be assessed by combining the candidate inhibitor with the protein, a substrate of its enzymatic activity (e.g., histones) acetyl CoA (e.g., radiolabelled acetyl CoA) and other assay components (e.g., an appropriate physiological solution or buffer), to produce a combination. The combination is maintained under conditions under which the enzymatic activity of the protein is maintained and appropriate for the protein to act upon/interact with its substrate (e.g., for the CDY gene to retain its histone acetyltransferase activity). As a result, the substrate is acted upon by the protein if the candidate inhibitor does not inhibit the protein and the protein acts upon the substrate. If the substrate is not acted upon by the protein, this is an indication that the candidate inhibitor is an inhibitor of the protein. For example, if a histone acetyltransferase, such as CDY-encoded protein is inhibited by a candidate inhibitor, its histone acetyltransferase activity will be blocked. If radiolabelled acetyl CoA is used, transfer of the radiolabelled acetyl group to the enzyme substrate (histones) is inhibited (will not occur or

will occur to a lesser extent than occurs in the absence of the candidate inhibitor). Whether transfer occurs can be assessed by determining the location of radiolabelled acetyl groups from acetyl CoA. If the histone substrates are not radiolabelled or are radiolabelled to a lesser extent in the presence of a candidate inhibitor (than in its absence), the candidate inhibitor is an inhibitor of the protein. Inhibitors identified in this way can be further assessed in additional *in vitro* assays or in *in vivo* assays (e.g., in an appropriate animal model).

To interpret the observation that these X-homologous and multi-copy, testis-specific groups account for 18 of 20 known NRY genes or families, we postulate that the NRY's evolution was dominated by two strategies. The first strategy favors conservation of certain existing genes and the second favors the acquisition of a class of novel genes: 1) The X-homologous genes probably reflect the common ancestry of the X and Y chromosomes, and selective pressures to maintain comparable expression of genes in males and females. 2) The abundance of testis-specific families may have resulted from the NRY's selectively retaining and amplifying genes that enhance male reproductive fitness.

1) Dosage compensation and X-Y homology. Experts agree that the mammalian X and Y chromosomes evolved from autosomes, with nearly all ancestral gene functions deteriorating on the non-recombining portion of the emerging Y chromosome while being maintained on the nascent X chromosome (J. J. Bull, *Evolution of Sex Determining Mechanisms* (Benjamin Cummings, Menlo Park, CA, 1983); J. A. Graves, *Annu. Rev. Genet. 30*:233 (1996); B. Charlesworth, *Curr. Biol. 6*:149 (1996); W. R. Rice, *BioScience 46*:331 (1996)). Functional degeneration of the NRY would result in females having two, but males only one, copy of many genes, creating the need for a mechanism to equalize

X-linked gene expression in the sexes. In mammals, a predominant solution to this problem is provided by X inactivation, the transcriptional silencing of one X chromosome in females.

However, the findings on X-homologous NRY genes described herein, combined with previous studies, illustrate the importance in human evolution of an alternative solution: preservation of homologous genes on both the NRY and the X chromosome, with both male and female cells expressing two copies of such genes. A critical prediction of this model is that, in female cells, the X homologs should escape X inactivation. This is the case for all widely expressed X-linked genes with known NRY homologs, including the X homologs of five novel NRY genes reported here (E. M. Fisher, *et al.*, *Cell 63*:1205 (1990); A. I. Agulnik*et al.*, *Hum. Mol. Genet. 3*:879 (1994); M. H. Jones *et al.*, *Hum. Mol. Genet. 5*:1695 (1996); J. A. Fischer-Vize *et al.*, *Development 116*:985 (1992); Y. Huang *et al.*, *Science 270*:1828 (1995); A. Schneider-Gädicke *et al.*, *Cell 57*:1247 (1989)). A second prediction of this model is that the human X and Y encoded proteins should be functionally interchangeable even though the nucleotide sequences of their corresponding genes are considerably diverged. Indeed, each of the eight known X-NRY gene pairs encode closely related isoforms, with 83 to 97% amino acid identity throughout their lengths; functional interchangeability has been demonstrated in the one case tested to date (M. Watanabe *et al.*, *Nat. Genet. 4*:268 (1993)).

Turner syndrome is classically associated with an XO sex chromosome constitution. In 1965, Ferguson-Smith postulated that the Turner phenotype might be due to inadequate expression of X-Y common genes that escape X inactivation (M. A. Ferguson-Smith, *J. Med. Genet. 2*:142 (1965)). These "Turner genes" have yet to be identified

with certainty. However, there now exists a substantial collection of X-homologous NRY genes (Figure 1) which can be assessed for genes which contribute to or are responsible for the Turner phenotype. The potential role of *RPS4Y* and *RPS4X* in Turner syndrome is controversial (E. M. Fisher *et al.*, *Cell 63:*1205 (1990); W. Just *et al.*, *Hum. Genet. 89:*240 (1992)). At least one Turner gene maps to the Xp-Yp pseudoautosomal region (T. Ogata *et al.*, *J. Med. Genet. 30:*918 (1993)). Seven of the eight known X-NRY gene pairs appear to be ubiquitously expressed, and at least three encode housekeeping proteins: an essential ribosomal protein (RPS4), an essential translation initiation factor (eIF-1A), and a modulator of actin polymerization (thymosin ß4). Perhaps some features of the XO phenotype (e.g., poor fetal viability) reflect inadequate expression of such housekeeping functions.

2) Male fitness and Y-specific, testis-specific genes. As first appreciated by R.A. Fisher, animal genomes may contain genes or alleles that enhance male reproductive fitness but are inconsequential or detrimental with respect to female fitness (R. A. Fisher, *Biol. Rev. 6:*345 (1931)). As Fisher recognized, selective pressures would tend to favor the accumulation of such genes in male-specific regions of genomes. Of course, male reproductive fitness depends critically on sperm production, the central task of the adult testis. Since the NRY is the only male-specific portion of the mammalian genome, it should have a unique tendency to accumulate male-benefit genes during evolution.

These principles are illustrated by several gene families on the human NRY. *De novo* deletions of the *DAZ* gene cluster on the human Y chromosome are associated with severe spermatogenic defects (R. Reijo *et al.*, *Nat. Genet. 10:*383 (1995)), and in *Drosophila* the *DAZ* homolog *boule* is required for spermatogenesis (C. G. Eberhart *et al.*, *Nature 381:*783 (1996)). The *DAZ* gene cluster on the human Y

chromosome arose, during primate evolution, by transposition and amplification of an autosomal gene. Likewise, two other testis-specific NRY gene families —*YRRM* and *TSPY* — may also be the result of the Y chromosome's having acquired and amplified autosomal genes (R. Saxena *et al.*, *Nat. Genet. 14:*292 (1996); M. L. Delbridge *et al.*, *Nat. Genet. 15:*131 (1997)). It is possible that the selective advantage conferred by the NRY's retaining and amplifying male fertility factors (from throughout the genome) accounts for the multitude of testis-specific gene families there. This may have been the preeminent force in shaping the NRY's gene repertoire, as it appears that the great majority of NRY transcription units are members of such testis-specific families. In the NRY, each of the testis-specific gene families has multiple members, 20 to 40 copies in the case of *TSPY* (E. Manz *et al.*, *Genomics 17:* 726 (1993)), and perhaps as many as 20 copies in the case of *YRRM* (K. Ma *et al.*, *Cell 75:*1287 (1993)). All together, the various Y-specific gene families may include as many as several hundred genes or copies. Though it is not known how many of these are functional, it seems likely that Y-specific, testis-specific gene families comprise the great majority of NRY transcription units.

Recent genetic studies underscore the importance of the human Y chromosome in fertility. Many men with spermatogenic failure, but who are otherwise healthy, have deletions of portions of the NRY (K. Ma *et al.*, *Cell 75:* 1287 (1993); R. Reijo *et al.*, *Nat. Genet. 10:*383 (1995); P. H. Vogt *et al.*, *Hum. Mol. Genet. 5:*933 (1996); J. L. Pryor *et al.*, *New England J. Med. 336:*534 (1997)). These findings suggested the existence of NRY genes that play critical roles in male germ cell development but are not required elsewhere in the body. Previous deletion mapping studies have implicated four regions of the NRY in either

spermatogenic failure or germ cell tumorigenesis, and in each of these four regions we now report novel candidate genes expressed specifically, or most abundantly, in testes (Figure 1). As shown in Figure 1, the region implicated in gonadoblastoma, stature and spermatogenic failure all contain novel candidate genes. Two of the three regions implicated in spermatogenic failure each contain one or more novel testis-specific genes. The third region implicated in spermatogenic failure (intervals 5B-5D) contains two X-homologous genes, *DBY* and *EIF1AY*, with abundant, testis-specific transcripts in addition to higher-molecular-weight, ubiquitous transcripts.

While X-homologous and testis-specific genes are somewhat intermingled within the NRY, clustering is evident (Figure 1). The geographic distribution of the two classes correlates quite well with previously identified sequence domains within the euchromatic NRY (D. Vollrath *et al.*, *Science 258:*52 (1992); S. Foote *et al.*, *Science 258:*60 (1992)). Ten of the 11 known testis-specific families map to previously identified regions of Y-specific repetitive sequences. The only exception is *BPY1*, which cross-hybridizes to the X chromosome and maps to a previously recognized region of X homology. Indeed, one or more testis-specific gene families are found in nearly all known regions of euchromatic Y repeats (Figure 1). Ironically, it had been widely assumed that these regions consisted of "junk" DNA, partly on theoretical grounds (B. Charlesworth, *Science 251:*1030 (1991); E. Seboun *et al.*, *Cold Spring Harb. Symp. Quant. Biol. 1:*237 (1986)). To the contrary, the results presented here argue that these Y-specific repetitive regions contain the great majority of the NRY's transcription units (The only exception is *BPY1*, which cross-hybridizes to the X chromosome and maps to a previously recognized region of X homology). These regions may be the result of rampant gene amplification during

mammalian evolution. By contrast, none of the eight X-homologous genes map to the Y-repeat regions; all eight map to regions previously identified as consisting largely of single-copy (or in some cases X-homologous) sequences. It is possible that, early in mammalian evolution, these regions of the NRY shared extensive sequence identity with the nascent X chromosome. The stage is now set for systematic evolutionary, biochemical and cell biological studies of the NRY, an idiosyncratic segment of the human genome.

The present invention relates to isolated DNA and genes, present on (which occur on) the Y chromosome, whose sequences are provided herein, as well as characteristic portions of the DNA. It relates to additional nucleic acid/nucleotide sequences which are not identical to the sequences presented herein but include substitutions or differences; DNA which includes substitutions or differences and encodes the same amino acid sequence as a DNA whose sequence is provided herein or includes substitutions which do not alter the ability of a DNA probe or primer which hybridizes to DNA whose sequence is presented herein to hybridize to the DNA containing the substitutions or differences. It further relates to DNA which encodes a protein or peptide whose sequence is presented herein. The present invention also includes the complements of the DNA sequences presented herein, DNA which hybridizes under stringent (high stringency) conditions to the DNA whose sequences are presented and to RNA transcripts. The invention further relates to encoded proteins, peptides and other products (e.g., glycoproteins) and antibodies which are raised against or bind to proteins or peptides whose amino acid sequences are presented herein or are encoded by DNA whose sequences are provided. As used herein, the term isolated DNA which occurs on the non-recombining region of the human Y chromosome refers to DNA

which has been obtained or removed from the human Y chromosome or DNA, produced by any means (e.g., recombinant techniques, synthetic methods), which has the sequence of such Y chromosome DNA. For example, isolated testis-specific DNA or isolated testis-specific DNA which occurs on the non-recombining region of the human Y chromosome is DNA which has been obtained or removed from the non-recombining region of the human Y chromosome or which has the sequence of such DNA and has been obtained or produced by any means.

Thus, this invention has application to several areas. It may be used diagnostically to identify males with reduced sperm count in whom a gene has been deleted or altered. It may also be used therapeutically in gene therapy treatments to remedy fertility disorders associated with deletion or alteration of a gene described. In one embodiment of a gene therapy method, a gene described herein, or a gene portion which encodes a functional protein, is introduced into a man whose sperm count is reduced and in whom the gene is expressed and the encoded protein replaces the protein normally produced or enhances the quantity produced. The present invention may also be useful in designing or identifying agents which function as a male contraceptive by inducing reduced sperm count. This invention also has application as a research tool, as the nucleotide sequences described herein have been localized to regions of the Y chromosome.

The present invention includes nucleotide sequences described herein, and their complements, which are useful as hybridization probes or primers for an amplification method, such as polymerase chain reaction (PCR), to show the presence, absence or disruption of the gene of the present invention. Probes and primers can have all or a portion of the nucleotide sequence (nucleic acid sequence) of a gene described herein or all or a portion of its

complement. For example, sequences shown in the Figures or Example 2 (SEQ ID NOS.: 1-84), as well as the complements thereof, can be used. The probes and primers can be any length, provided that they are of sufficient length and appropriate composition (appropriate nucleotide sequence) to hybridize to all or an identifying or characteristic portion of the gene described or to a disrupted form of the gene, and remain hybridized under the conditions use. Useful probes include, but are not limited to, nucleotide sequences which distinguish between a gene described herein and an altered form of that gene shown to be associated with reduced sperm count (azoospermia, oligospermia). Generally, the probe will be at least 7 nucleotides, while the upper limit is the length of the gene itself, e.g., up to about 40,000 nucleotides in length. Probes can be, for example, 10 to 14 nucleotides or longer (e.g., 20, 30, 50, 100, 250 nucleotides or any other useful length); the length of a specific probe will be determined by the assay in which it is used.

In one embodiment, the present invention is a method of diagnosing or aiding in the diagnosis of reduced sperm count associated with deletion or alteration of a gene described herein. Any man may be assessed with this method of diagnosis. In general, the man will have been at least preliminarily assessed, by another method, as having a reduced sperm count. By combining nucleic acid probes derived either from the isolated native sequence or cDNA sequence of the gene, or from appropriate primers, with the DNA from a sample to be assessed, under conditions suitable for hybridization of the probes with unaltered complementary nucleotide sequences in the sample but not with altered complementary nucleotide sequences, it can be determined whether the man possesses the intact gene. If the gene is unaltered, it may be concluded that the alteration of the gene is not responsible for the reduced

sperm count. This invention may also be used in a similar method wherein the hybridization conditions are such that the probes will hybridize only with altered DNA and not with unaltered sequences. The hybridized DNA can also be isolated and sequenced to determine the precise nature of the alteration associated with the reduced sperm count. DNA assessed by the present method can be obtained from a variety of tissues and body fluids, such as blood or semen. In one embodiment, the above methods are carried out on DNA obtained from a blood sample.

The invention also provides expression vectors containing a nucleotide (nucleic acid) sequence described herein, which is operably linked to at least one regulatory sequence. "Operably linked" is intended to mean that the nucleotide sequence is linked to a regulatory sequence in a manner which allows expression of the nucleotide sequence. The term "regulatory sequence" included promoters, enhancers, and other expression control elements (see, e.g., Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the protein or peptide desired to be expressed. For instance, the peptides of the present invention can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells or both (see, for example, Broach, *et al.*, Experimental Manipulation of Gene Expression, ed. M, Inouye (Academic Press, 1983) p. 83; Molecular Cloning: A Laboratory Manual, 2nd Ed., ed. Sambrook *et al.* (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17).

Prokaryotic and eukaryotic host cells transfected by the described vectors are also provided by this invention. For instance, cells which can be transfected with the

vectors of the present invention include, but are not limited to, bacterial cells such as *E. coli*, insect cells (baculovirus), yeast and mammalian cells, such as Chinese hamster ovary cells (CHO).

Thus, a nucleotide sequence described herein can be used to produce a recombinant form of the protein via microbial or eukaryotic cellular processes. Production of a recombinant form of the protein can be carried out using known techniques, such as by ligating the oligonucleotide sequence into a DNA or RNA construct, such as an expression vector, and transforming or transfecting the construct into host cells, either eukaryotic (yeast, avian, insect or mammalian) or prokaryotic (bacterial cells). Similar procedures, or modifications thereof, can be employed to prepare recombinant proteins according to the present invention by microbial means or tissue-culture technology.

The present invention also pertains to pharmaceutical compositions comprising the proteins and peptides described herein. For instance, the peptides or proteins of the present invention can be formulated with a physiologically acceptable medium to prepare a pharmaceutical composition. The particular physiological medium may include, but is not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polethylene glycol) and dextrose solutions. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to medicinal chemists, and will depend on the ultimate pharmaceutical formulation desired. Methods of introduction of exogenous polypeptides at the site of treatment include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral and intranasal. Other suitable methods of introduction can also include rechargeable or biodegradable devices and slow release polymeric devices. The

pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

This invention also has utility in methods of treating disorders of reduced sperm count associated with deletion or alteration of a gene described herein. These genes may be used in a method of gene therapy, whereby the gene or a gene portion encoding a functional protein is inserted into cells in which the functional protein is expressed and from which it is generally secreted to remedy the deficiency caused by the defect in the native gene.

The present invention is also related to antibodies which bind a protein or peptide encoded by all or a portion of a gene of the present invention, as well as antibodies which bind the protein or peptide encoded by all or a portion of a disrupted form of the gene. For instance, polyclonal and monoclonal antibodies which bind to the described polypeptide or protein are within the scope of the invention. A mammal, such as a mouse, hamster or rabbit, can be immunized with an immunogenic form of the protein or peptide (an antigenic fragment of the protein or peptide which is capable of eliciting an antibody response). Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques are well known in the art. The protein or peptide can be administered in the presence of an adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibody.

Following immunization, anti-peptide antisera can be obtained, and if desired, polyclonal antibodies can be isolated from the serum. Monoclonal antibodies can be isolated from the serum. Monoclonal antibodies can also be produced by standard techniques which are well known in the

art (Koehler and Milstein, Nature 256: 495-497 (19775); Kozbar *et al.*, Immunology Today 4: 72 (1983); and Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)). Such antibodies are useful as diagnostics for the intact or disrupted gene and also as research tools for identifying either the intact or disrupted gene.

The present invention is illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLE 1 ISOLATION OF CDNA CLONES FROM HUMAN TESTIS LIBRARY

"cDNA selection" (M. Lovett *et al.*, *Proc. Natl. Acad. Sci. USA 88:*9628 (1991)) was carried out using bulk cDNA prepared from human adult testes (Clontech, Palo Alto, CA) and, as selector, a cosmid library prepared from flow-sorted Y chromosomes (Lawrence Livermore National Laboratory: LL0YNC03). A total of 3600 random cosmids, providing nearly five-fold coverage of the 30-Mb euchromatic region, were used to generate 150 pools of selector DNA. Using each of the 150 selector pools, we carried out four successive rounds of cDNA selection, followed by two rounds of subtraction with human COT-1 DNA (Gibco BRL, Gaithersburg, MD) to remove highly repetitive sequences. A plasmid library was prepared from each of the 150 resulting pools of selected cDNA fragments, and 24 clones from each library were sequenced from one end. Of the 3600 sequences generated, about 600 were of poor technical quality and about 500 were found to derive from cloning vector or *E. coli* host, leaving 2539 sequences for further analysis. Of the 2539 sequence fragments, 536 corresponded to previously reported NRY genes (487 to *TSPY*, 15 to *YRRM*, 14 to *RPS4Y*, 9 to *SMCY*, 5 to *DAZ*, 3 to *SRY*, 3 to *ZFY*) and 41 corresponded to previously reported pseudoautosomal genes (15 to *XE7*, 11 to *CSF2RA*, 4 to *IL3RA*,

3 to *ASMT*, 3 to *IL9R*, 2 to *ANT3*, 2 to *MIC2*, 1 to *SYBL1*). Electronic analysis of the roughly 2000 remaining sequences revealed that about 200 contained known repetitive elements, and these were not pursued. By electronically identifying redundancies and sequence overlaps, the remaining sequences were reduced to 1093 sequence contigs. Sequences representing these 1093 contigs were individually hybridized to dot-blotted yeast genomic DNAs of 60 YACs comprising most of the Y's euchromatic region (S. Foote *et al.*, *Science 258:*60 (1992)). 181 sequences that hybridized to the great majority of the YACs were judged likely to contain highly repeated elements and were not pursued, leaving 912 sequences for further analysis. The 912 sequences were individually hybridized to Southern blots of R1-digested human 46,XX female and 49,XYYYY male (L. Sirota *et al.*, *Clin. Genet. 19:*87 (1981)) genomic DNAs. Blots were hybridized at 65°C in Church's buffer (0.5 M Na_2PO_4 at pH7.5, with 7% SDS), and washed at 65°C in 1X SSC and 0.1% SDS, with 832 hybridizations yielding interpretable results. Many sequences appeared to contain highly repeated elements common to males and females, or failed to detect an unambiguously Y-specific restriction fragment, and these were not pursued. By contrast, 308 sequences hybridized to at least one prominent fragment present in 49,XYYYY but absent in 46,XX, suggesting that these sequences derived from the NRY. Each of these 308 sequences was individually used to screen, by hybridization, about 2 million plaques from a 1 phage library of human adult testis cDNA (Clontech, Palo Alto, CA).

EXAMPLE 2 LOCALIZATION OF 12 NOVEL GENES ON THE Y CHROMOSOME

Genes were localized on a previously reported NRY deletion map by testing with PCR for their presence or

absence in individuals carrying partial Y chromosomes (D. Vollrath *et al.*, *Science 258:*52 (1992)). Most genes were localized to a single deletion interval. Some genes could not be unambiguously placed because copies exist in multiple locations in the NRY. In such cases, genes were localized by PCR testing of YACs encompassing the NRY's euchromatic region (S. Foote *et al.*, *Science 258:*60 (1992)). X homologs of Y genes were mapped onto the X by PCR testing a panel of human/rodent somatic hybrid cell lines (Research Genetics, Huntsville, AL). All PCR assays consists of 30 cycles of the following conditions: 1 min denaturing at 94°C, 45 sec annealing at 60°C, and 45 sec extension at 72°C. *TB4X* primers were designed from an unreported intron. TPRX primers were designed from unreported cDNA sequence. All other primers were designed from cDNA sequences as submitted to Genbank. PCR primers were as follows:

GENE	LEFT PRIMER	RIGHT PRIMER
DBY	CATTCGGTTTTACCAGCCAG	CAGTGACTCGAGGTTCAATG
	(SEQ ID NO.: 51)	(SEQ ID NO.: 52)
TPRY	GCATCATAATATGGATCTAGTAGG	GGAGATACTGAATAGCATAGC
	(SEQ ID NO.: 53)	(SEQ ID NO.: 54)
TB4Y	CAAAGACCTGCTGACAATGG	CTCCGCTAAGTCTTTCACC
	(SEQ ID NO.: 55)	(SEQ ID NO.: 56)
EIF1AY	CTCTGTAGCCAGCCTCTTC	GACTCCTTTCTGGCGGTTAC
	(SEQ ID NO.: 570	(SEQ ID NO.: 58)
DFFRY	GAGCCCATCTTTGTCAGTTTAC	CTGCCAATTTTCCACATCAACC
	(SEQ ID NO.: 59)	(SEQ ID NO.: 60)
CDY	GGCTCAAAATCCACTGACG	CAAGCGATATCTCACCACC
	(SEQ ID NO.: 61)	(SEQ ID NO.: 62)
BPY1	CTCCCTGAGCAGCAACTAAG	GTCATCAACATGGGAAGCAC
	(SEQ ID NO.: 63)	(SEQ ID NO.: 64)
BPY2	CCAGGACCATGTGATATGG	CTAATTCCCTCTTTACGCATGACC
	(SEQ ID NO.: 65)	(SEQ ID NO.: 66)

XKRY	CACTCATGGAGAAGGGTAGG	GTCACACTCAGCCTCTTTAC
	(SEQ ID NO.: 67)	(SEQ ID NO.: 68)
PTPRY	GAGCACACCACACCAGAAAC	CTCAGACTGACCTCGGACTG
	(SEQ ID NO.: 69)	(SEQ ID NO.: 70)
TTY1	CTCTGGGAATCAAATTCGAGG	GTCTTTCAGCCAATCCAAGG
	(SEQ ID NO.: 71)	(SEQ ID NO.: 72)
TTY2	GACAACTCTGACAGCCAGG	GTCAGAACTCCCAAACAGG
	(SEQ ID NO.: 73)	(SEQ ID NO.: 74)
DBX	CTACATGCAGATGACATGGTG	GGCCAAGGTGCATAGGTG
	(SEQ ID NO.: 75)	(SEQ ID NO.: 76)
TPRX	CATGTTCCCTGTAGCACATC	CGTTTCCATTACTTCCATTTCCTG
	(SEQ ID NO.: 77)	(SEQ ID NO.: 78)
TB4X	CCCGCCCTTTCATCATCC	GCTCCCCAAAGTAGCCTTC
	(SEQ ID NO.: 79)	(SEQ ID NO.: 80)
EIF1AX	CACGAGGCGCCATTTGCTG	CTGGAGGCCAGGCAACGTG
	(SEQ ID NO.: 81)	(SEQ ID NO.: 82)
DFFRX	CCTCCACCTGAAGATGCC	CTGAGATCCAGGTGAATGG
	(SEQ ID NO.: 83)	(SEQ ID NO.: 84)

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

We claim:

1. Isolated testis-specific DNA which occurs on the non-recombining region of the human Y chromosome or the complement thereof.

2. The isolated testis-specific DNA of Claim 1 which occurs in multiple copies on the non-recombining region of the human Y chromosome or the complement thereof.

3. The isolated testis-specific DNA of Claim 2 selected from the group consisting of:

(a) a CDY gene or a characteristic portion thereof;
(b) a BPY 1 gene or a characteristic portion thereof;
(c) a BPY 2 gene or a characteristic portion thereof;
(d) an XKRY gene or a characteristic portion thereof;
(e) a PTPRY gene or a characteristic portion thereof;
(f) TTY1 DNA; or a characteristic portion thereof;
(g) TTY 2 DNA; or a characteristic portion thereof;
(h) a complement of (a);
(i) a complement of (b);
(j) a complement of (c);
(k) a complement of (d);
(l) a complement of (e);
(m) a complement of (f);
(n) a complement of (g);
(o) DNA encoding the amino acid sequence of SEQ ID No.: 39;.
(p) DNA encoding the amino acid sequence of SEQ ID No.: 40;
(q) DNA encoding the amino acid sequence of SEQ ID No.: 42;
(r) DNA encoding the amino acid sequence of SEQ ID No.: 44;

(s) DNA encoding the amino acid sequence of SEQ ID No.: 46;

(t) DNA encoding the amino acid sequence of SEQ ID No.: 48; and

(u) DNA which hybridizes to a DNA of any one of(a) through (t) under stringent conditions.

4. Isolated testis specific DNA selected from the group consisting of:

(a) DNA of SEQ ID No.: 37;

(b) DNA of SEQ ID No.: 38;

(c) DNA of SEQ ID No.: 41;

(d) DNA of SEQ ID No.: 43;

(e) DNA of SEQ ID No.: 45;

(f) DNA of SEQ ID No.: 47;

(g) DNA of SEQ ID No.: 49;

(h) DNA of SEQ ID No.: 50;

(i) DNA encoding the amino acid sequence of SEQ ID No.39;

(j) DNA encoding the amino acid sequence of SEQ ID No.40;

(k) DNA encoding the amino acid sequence of SEQ ID No.42;

(l) DNA encoding the amino acid sequence of SEQ ID No.44;

(m) DNA encoding the amino acid sequence of SEQ ID No.46;

(n) DNA encoding the amino acid sequence of SEQ ID No.48;

(o) a complement of a DNA of any one of (a) through (n); and

(p) DNA which hybridizes to a DNA of any one of (a) through (o) under stringent conditions.

5. Isolated X-homologous DNA which occurs on the non-recombining region of the human Y chromosome, is not testis-specific and has a homolog on the human X chromosome.

6. The isolated DNA of Claim 5 selected from the group consisting of:

(a a DBY gene or a characteristic portion thereof;
(b) a TPRY gene or a characteristic portion thereof;
(c) a TB4Y gene or a characteristic portion thereof;
(d) an EIF1AY gene or a characteristic portion thereof;
(e) a DFFRY gene or a characteristic portion thereof;
(f) a complement of (a);
(g) a complement of (b);
(h) a complement of (c);
(i) a complement of (d);
(j) a complement of (e);
(k) a complement of (f);
(l) DNA encoding the amino acid sequence of SEQ ID No.: 18;
(m) DNA encoding the amino acid sequence of SEQ ID No.: 22;
(n) DNA encoding the amino acid sequence of SEQ ID No.: 23
(o) DNA encoding the amino acid sequence of SEQ ID No.: 24;
(p) DNA encoding the amino acid sequence of SEQ ID No.: 28;
(q) DNA encoding the amino acid sequence of SEQ ID No.: 32;
(r) DNA encoding the amino acid sequence of SEQ ID No.: 36; and;

(s) DNA which hybridizes to a DNA of any one of (a) through (r) under stringent conditions.

7. Isolated X-homologous human DNA selected from the group consisting of:

(a) DNA of SEQ ID No.: 17 or a characteristic portion thereof;

(b) DNA of SEQ ID No.: 19 or a characteristic portion thereof;

(c) DNA of SEQ ID No.: 20 or a characteristic portion thereof;

(d) DNA of SEQ ID No.: 21 or a characteristic portion thereof;

(e) DNA of SEQ ID No.: 26 or a characteristic portion thereof;

(f) DNA of SEQ ID No.: 30 or a characteristic portion thereof;

(g) DNA of SEQ ID No.: 34 or a characteristic portion thereof;

(h) DNA encoding the amino acid sequence of SEQ ID No.: 18;

(i) DNA encoding the amino acid sequence of SEQ ID No.: 22;

(j) DNA encoding the amino acid sequence of SEQ ID No.: 23;

(k) DNA encoding the amino acid sequence of SEQ ID No.: 24;

(l) DNA encoding the amino acid sequence of SEQ ID No.: 28;

(m) DNA encoding the amino acid sequence of SEQ ID No.: 32;

(n) DNA encoding the amino acid sequence of SEQ ID No.: 36;

(o) a complement of a DNA of any one of (a) through (n); and

(p) DNA which hybridizes to a DNA any one of (a) through (o) under stringent conditions.

8. A DNA probe comprising all or a characteristic portion of DNA of Claim 4.

9. A DNA probe comprising all or a characteristic portion of DNA of Claim 7.

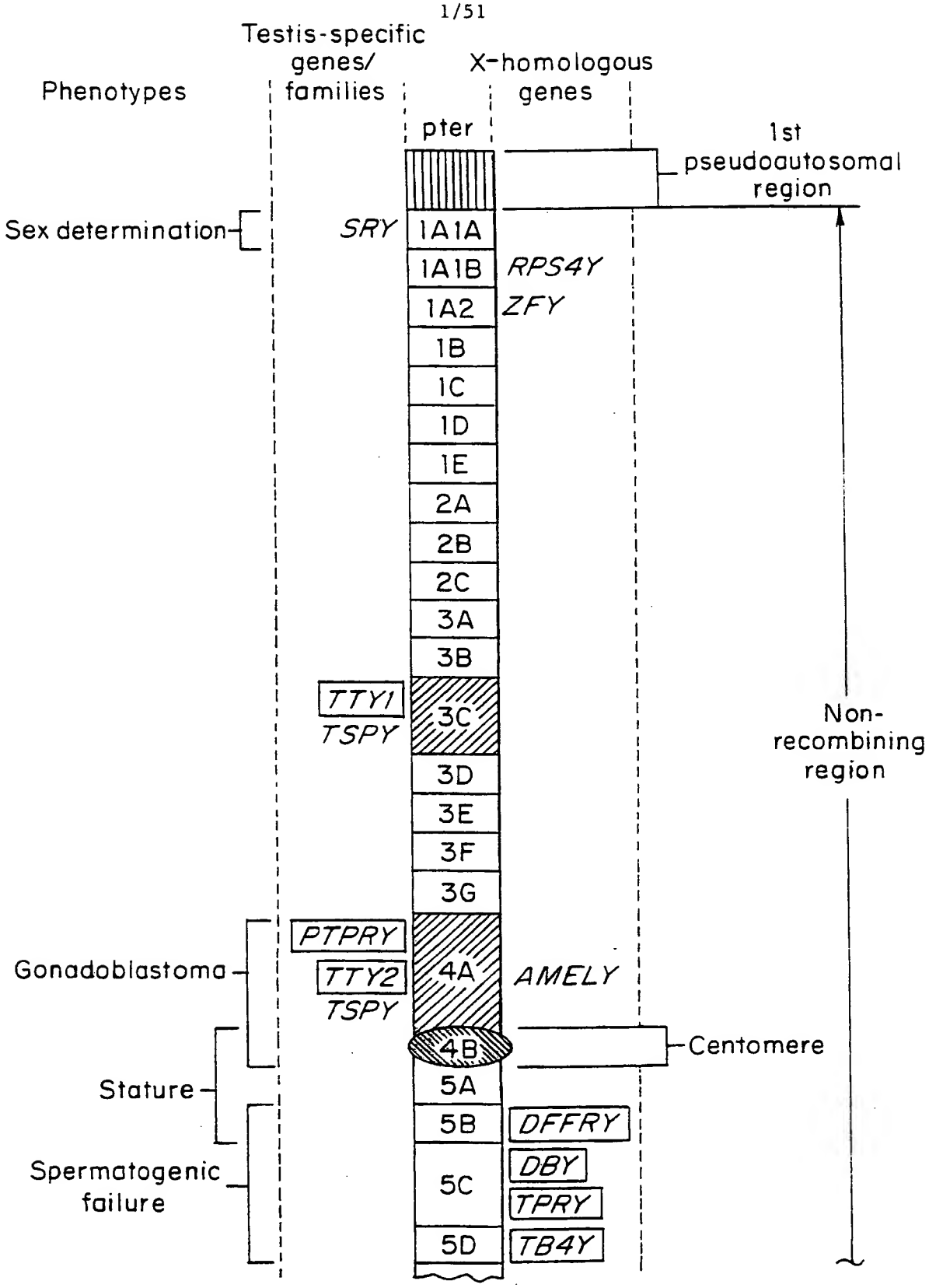

FIG. 1A

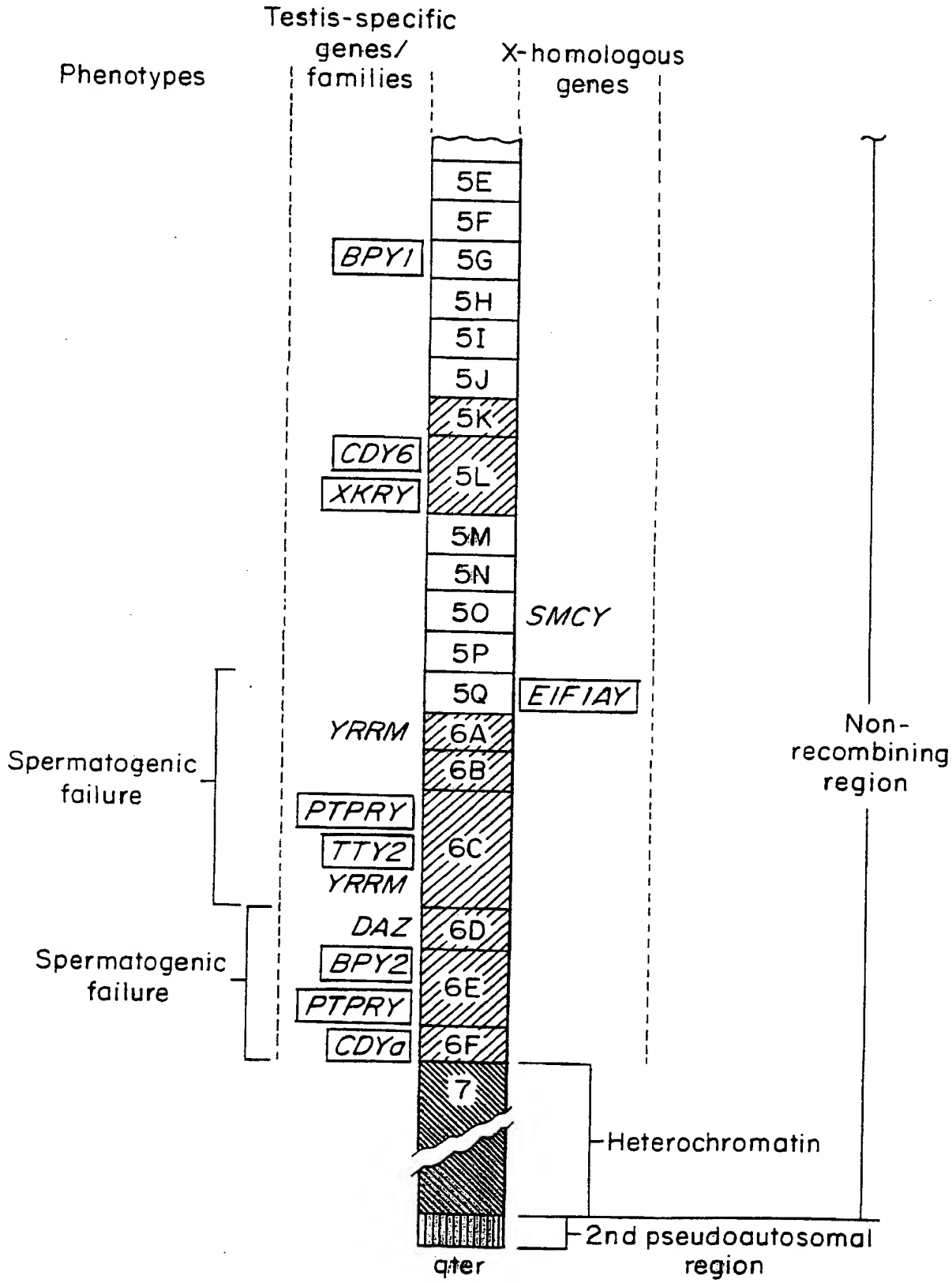

FIG. 1B

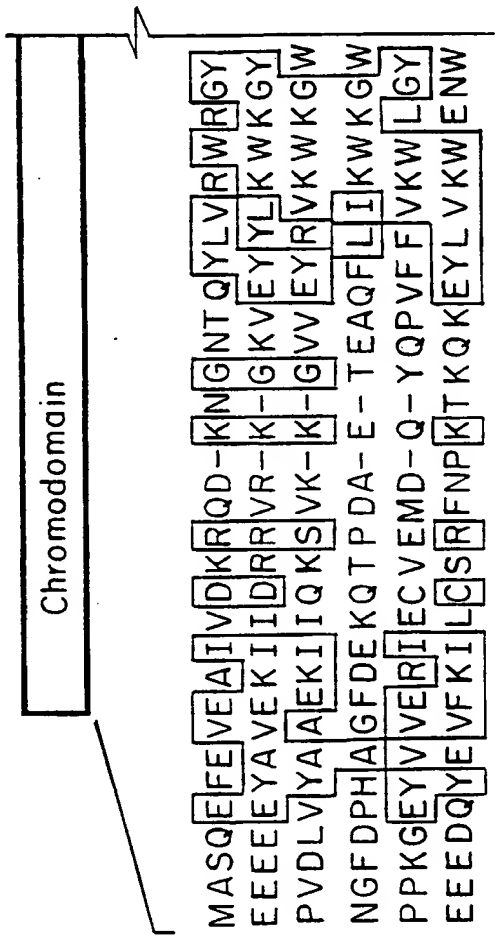

FIG. 2A

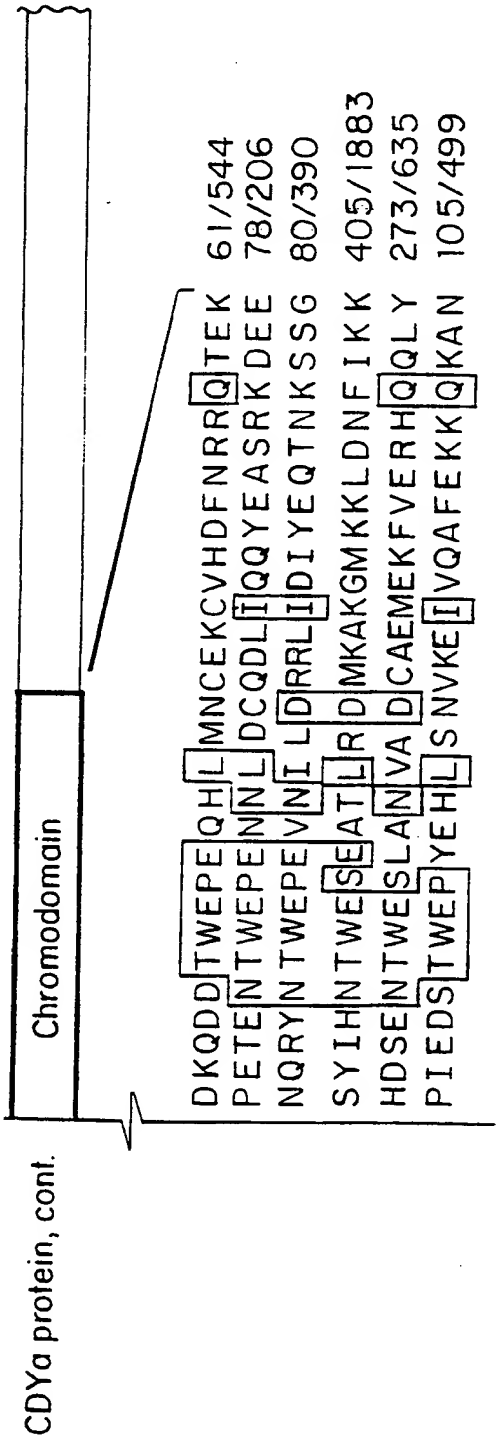

FIG. 2B

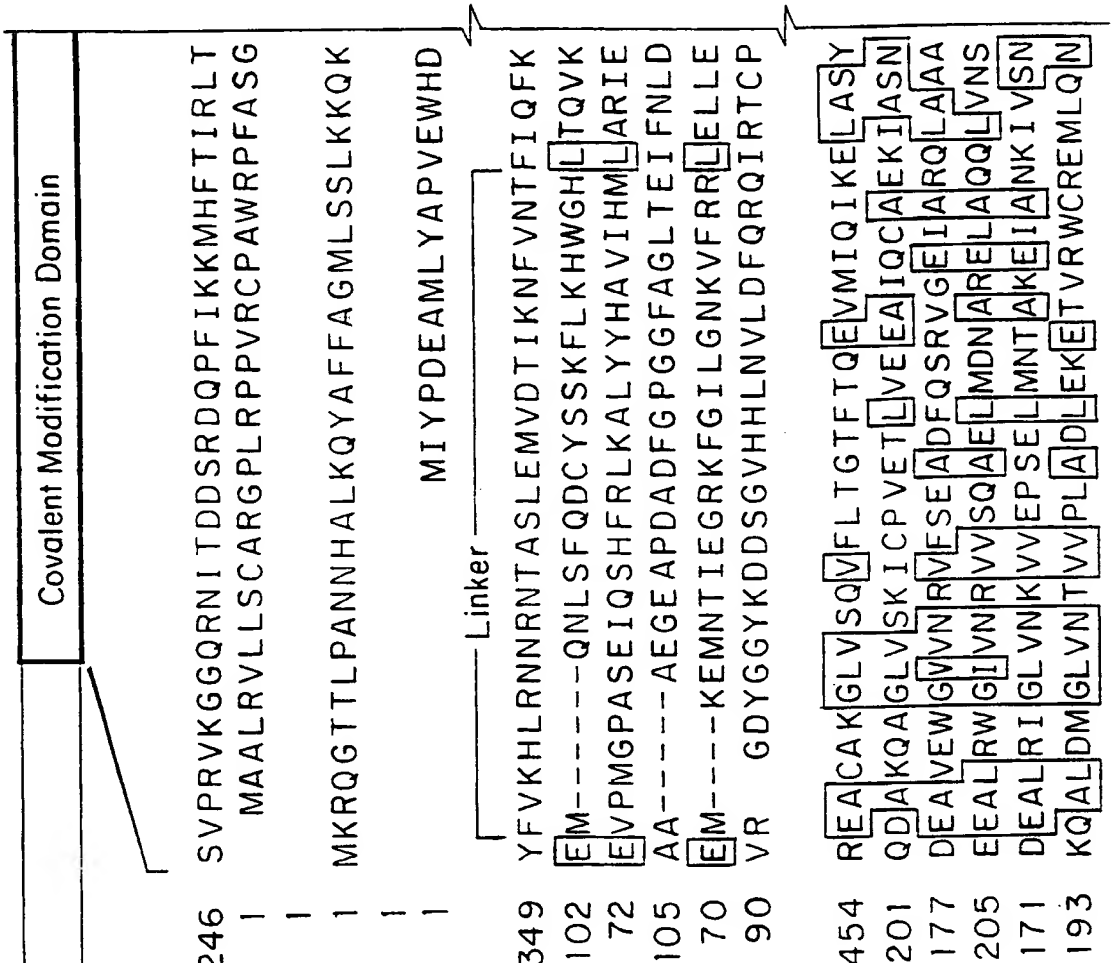

FIG. 2C

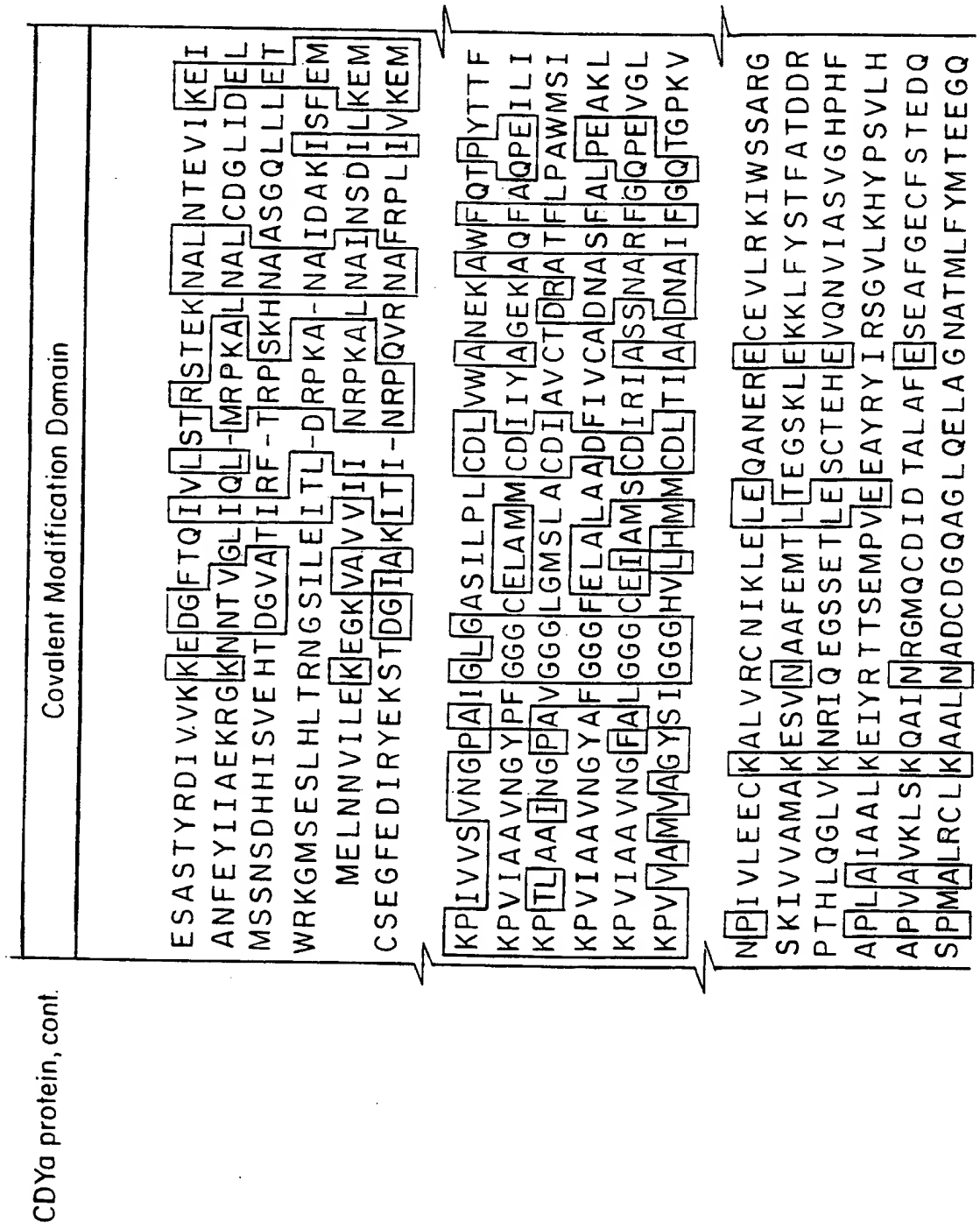

FIG. 2D

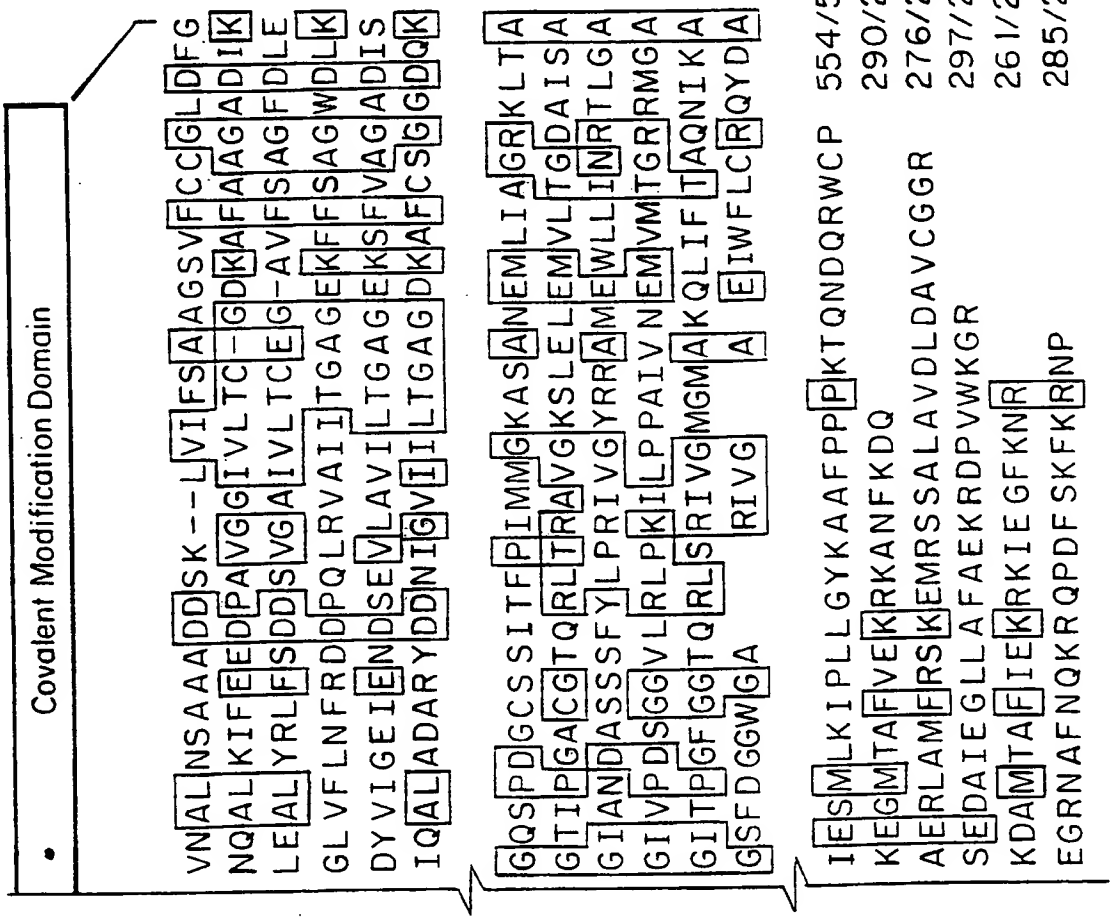

FIG. 2E

DBX & *DBY* short and long transcripts

```
DBX  -856                                                              ctttccccttactccgctcccctcttttccctccctctcctcccct
DBX  -810 tccctctgttctctcctcctcttcccctcccctcccccgtccggggcactctatattcaagccaccgtttcctgcttcacaaaatggcca
DBX  -720 ccgcacgcgacacctacggtcacgtggcctgccgccctctcagtttcgggaatctgcctagctcccactaaggggaggctacccgcggaa
DBX  -630 gagcgagggcagattagaccggagaaatcccaccacatctccaagcccgggaactgagagaggaagaagagtgaaggccagtgttaggaa
DBX  -540 aaaaaaaaacaaaaacaaaaaaaaacgaaaaacgaaagctgagtgcatagagttggaaaggggagcgaatgcgtaaggttggaaaggggg
DBX  -450 cgaagaggcctaggttaacattttcaggcgtcttagccggtggaaagcgggagacgcaagttctcgcgagatctcgagaactccgaggct
DBX  -360 gagactagggtttagcggagagcacgggaagtgtagctcgagagaactgggacagcatttcgcaccctaagctccaaggcaggactgct
DBX  -270 aggggcgacaggactaagtaggaaatcccttgagcttagacctgagggagcgcgcagtagccgggcagaagtcgccgcgacagggaattg
DBX  -180 cggtgtgagagggagggcacacgttgtacgtgctgacgtagccggctttccagcgggtatattagatccgtggccgcgcggtgcgctcca

DBX   -90 gagccgcagttctcccgtgag..g.ccttc.cggt.ga.caaaca...g.ttagcagcg.a.gact....g..c...g.a.........
DBY   -71                       ccagtgtaagagttccgctattcggtctcacacctacagtggactacccgattttcgcttctcttcaggg

       1  .   .   .   .   A   .   E   .   A   L   G   .   .   .   F   .   G   .   .   .   .   .   S   D   N   .   .   .   .
DBX    1  ..........CA..G.....CG.TC.GG...............T....GGC..A...............TCA..T..T.............
DBY    1  ATGAGTCATGTGGTGGTGAAAAATGACCCTGAACTGGACCAGCAGCTTGCTAATCTGGACCTGAACTCT---GAAAAACAGAGTGGAGGA
       1  M   S   H   V   V   V   K   N   D   P   E   L   D   Q   Q   L   A   N   L   D   L   N   S   -   E   K   Q   S   G   G

      30  -   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   R   .   .   T   R   .   .   Y   .   .   .   .   .   .
DBX   88  ---.......C...................T........T............CG.....TA...G....T...T.C..............G
DBY   88  GCAAGTACAGCGAGCAAAGGGCGCTATATACCTCCTCACTTAAGGAACAAAGAAGCATCTAAAGGATTCCATGATAAAGACAGTTCAGGT
      31  A   S   T   A   S   K   G   R   Y   I   P   P   H   L   R   N   K   E   A   S   K   G   F   H   D   K   D   S   S   G

      60  .   .   S   .   .   .   .   .   .   .   .   .   .   .   .   .   S   .   .   .   .   .   S   S   F   .   .   D   .   .
DBX  178  ......CT...............G...............A.....TAGT.....A.....G...T..A.C.TC.......T.......
DBY  178  TGGAGTTGCAGCAAAGATAAGGATGCATATAGCAGTTTTGGGTCTCGA---GATTCTAGAGGAAAGCCTGGTTATTTCAGTGAACGTGGA
      60  W   S   C   S   K   D   K   D   A   Y   S   S   F   G   S   R   -   D   S   R   G   K   P   G   Y   F   S   E   R   G

      89  .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   S   .   G   D   .   S   .   .   .   K   .   .
DBX  268  ................G.......................T..C......C........GC...GGT..C...AG.........A..
DBY  265  AGTGGATCAAGGGGAACATTTGATGATCGTGGACGGAGTGACTATGATGGTATTGGCAATCGT---GAAAGACCTGGCTTTGGCAGATTT
      90  S   G   S   R   G   R   F   D   D   R   G   R   S   D   Y   D   G   I   G   N   R   -   E   R   P   G   F   G   R   F

     118  .   .   G   .   N   .   .   .   .   .   .   D   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
DBX  358  .....TG.....A.C.....C...........A....A.......................C...................A..G...
DBY  352  GAACGGAGTGGACATAGTCGTTGGTGTGACAAGTCAGTTGAAGATGATTGGTCAAAACCACTTCCACCAAGTGAACGCTTGGAGCAAGAA
     120  E   R   S   G   H   S   R   W   C   D   K   S   V   E   D   D   W   S   K   P   L   P   P   S   E   R   L   E   Q   E
```

FIG. 3A

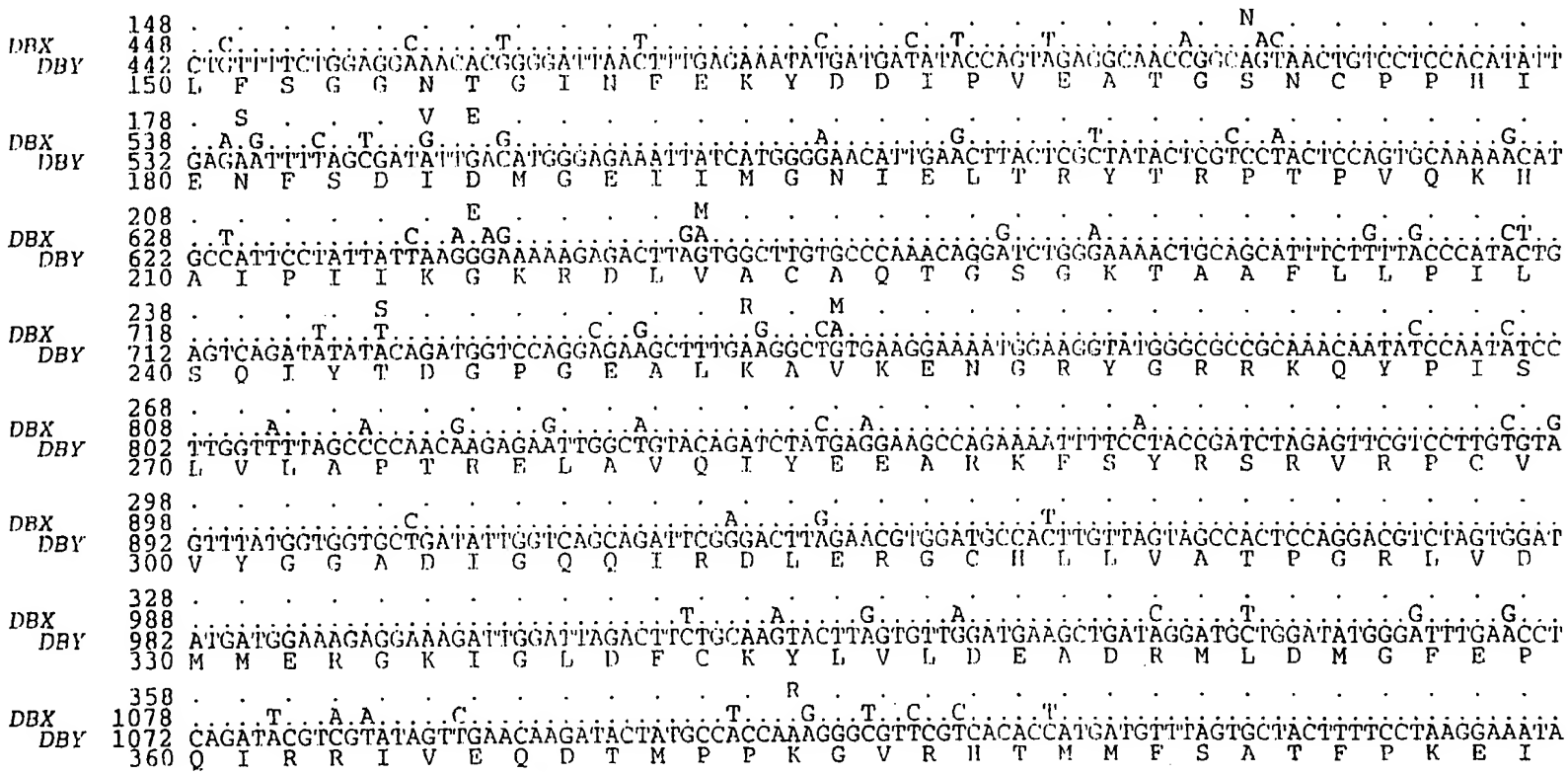

FIG. 3B

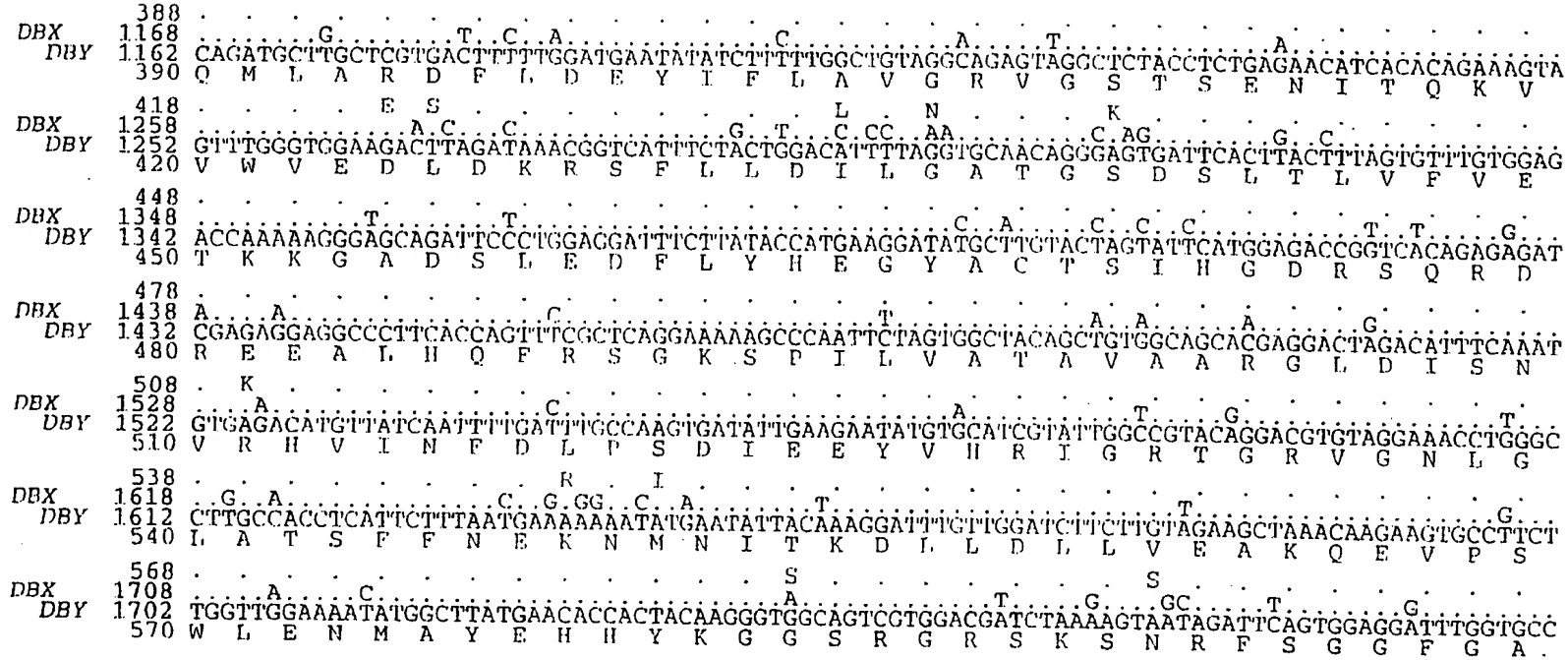

FIG. 3C

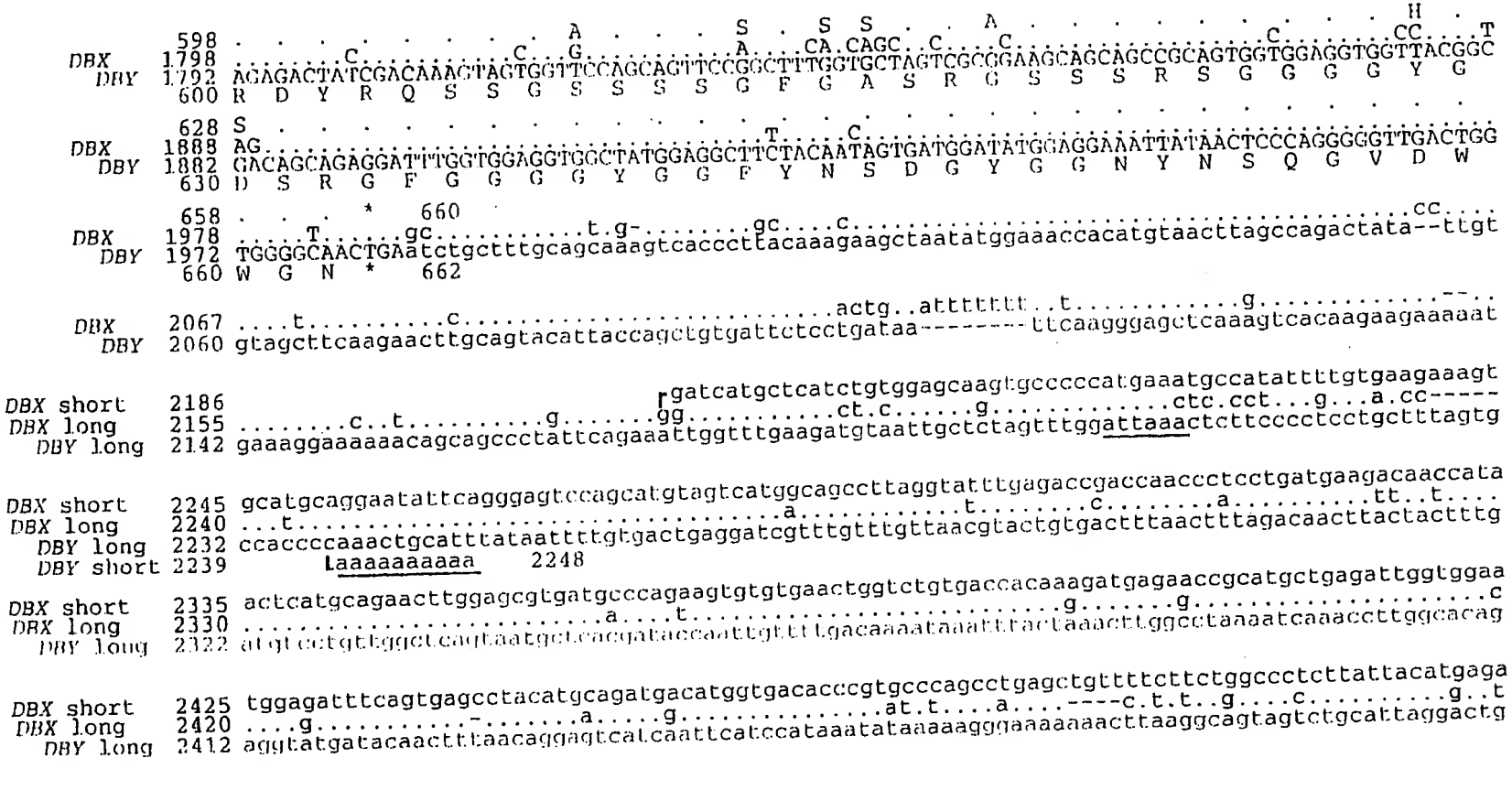

FIG. 3D

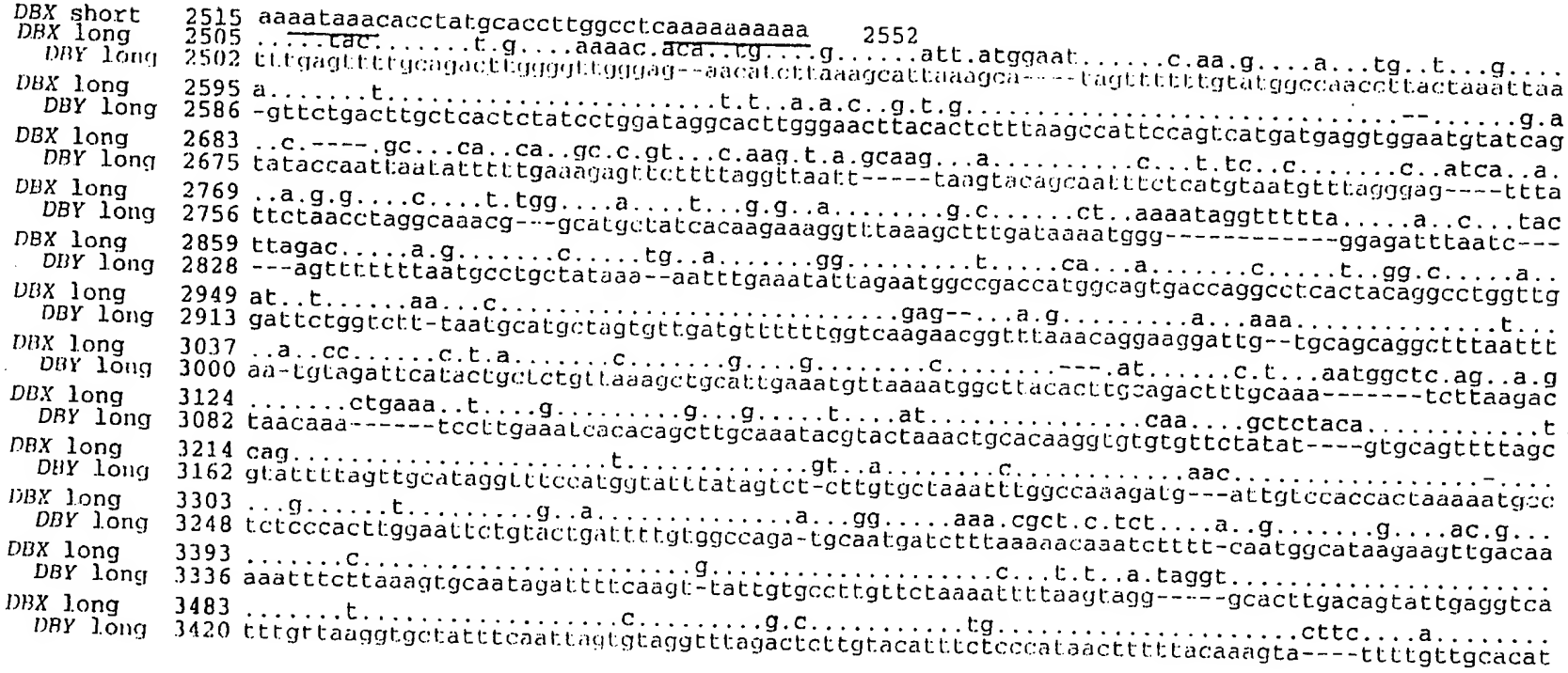

FIG. 3E

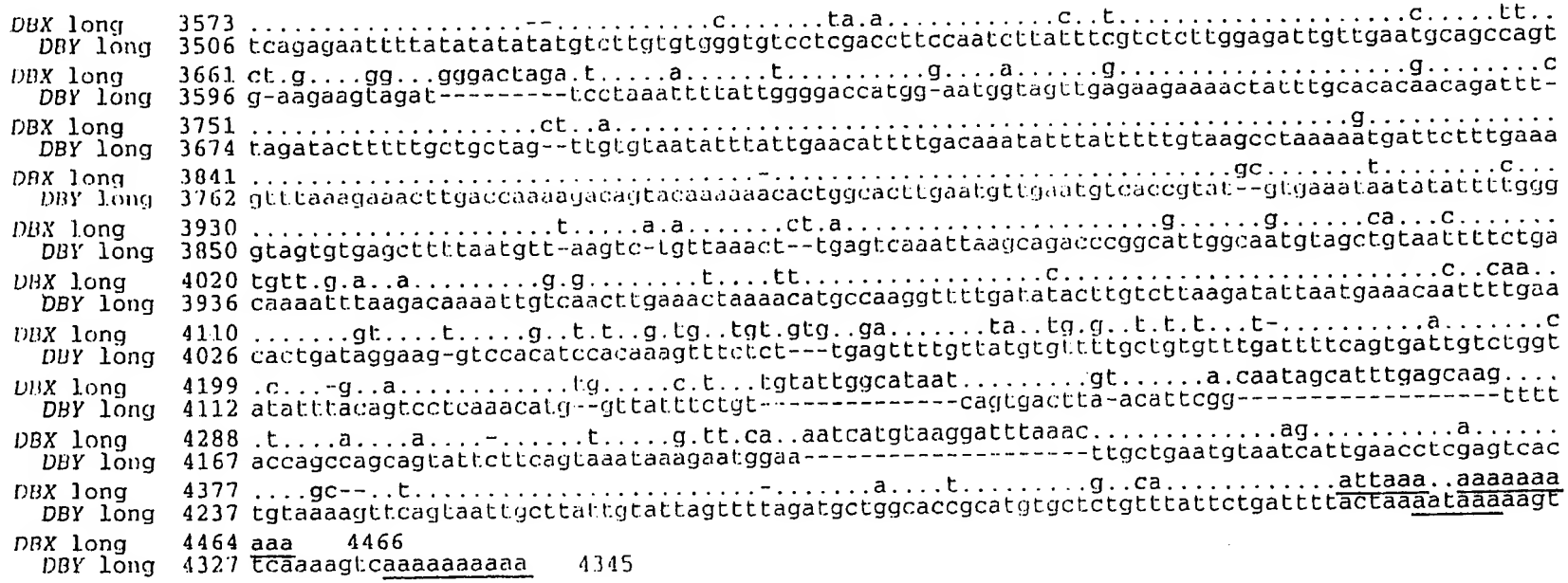

FIG. 3F

SUBSTITUTE SHEET (RULE 26)

TPRY
short, medium and long transcripts

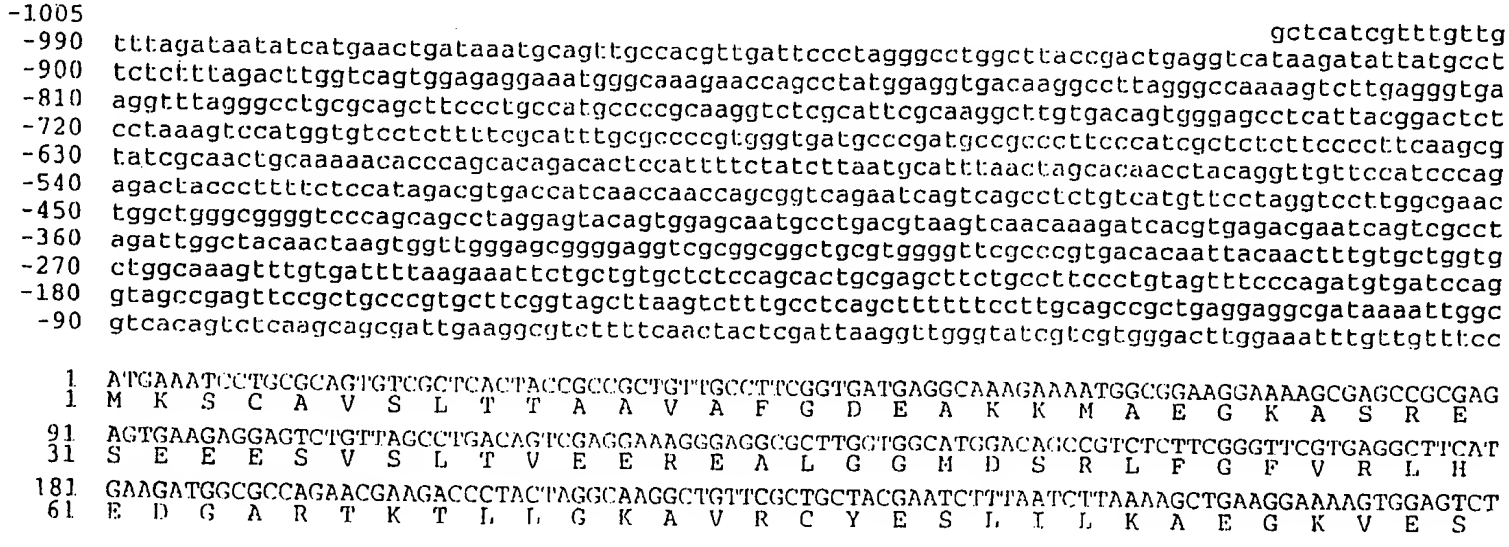

FIG. 4A

```
271  GACTTCTTTTGCCAATTAGGTCACTTCAACCTCTTGTTGGAAGATTATTCAAAAGCATTATCTGCATATCAGAGATATTACAGTTTACAG
 91  D  F  F  C  Q  L  G  H  F  N  L  L  L  E  D  Y  S  K  A  L  S  A  Y  Q  R  Y  Y  S  L  Q

361  GCTGACTACTGGAAGAATGCTGCGTTTTTATATGGCCTTGGTTTGGTCTACTTCTACTACAATGCATTTCATTGGGCAATTAAAGCATTT
121  A  D  Y  W  K  N  A  A  F  L  Y  G  L  G  L  V  Y  F  Y  Y  N  A  F  H  W  A  I  K  A  F

451  CAAGATGTCCTTTATGTTGACCCCAGCTTTTGTCGAGCCAAGGAAATTCATTTACGACTTGGGCTCATGTTCAAAGTGAACACAGACTAC
151  Q  D  V  L  Y  V  D  P  S  F  C  R  A  K  E  I  H  L  R  L  G  L  M  F  K  V  N  T  D  Y

541  AAGTCTAGTTTAAAGCATTTTCAGTTAGCCTTGATTGACTGTAATCCATGTACTTTGTCCAATGCTGAAATTCAATTTCATATTGCCCAT
181  K  S  S  L  K  H  F  Q  L  A  L  I  D  C  N  P  C  T  L  S  N  A  E  I  Q  F  H  I  A  H

631  TTGTATGAAACCCAGAGGAAGTATCATTCTGCAAAGGAGGCATATGAACAACTTTTGCAGACAGAAAACCTTCCTGCACAAGTAAAAGCA
211  L  Y  E  T  Q  R  K  Y  H  S  A  K  E  A  Y  E  Q  L  L  Q  T  E  N  L  P  A  Q  V  K  A

721  ACTGTATTGCAACAGTTAGGTTGGATGCATCATAATATGGATCTAGTAGGAGACAAAGCCACAAAGGAAAGCTATGCTATTCAGTATCTC
241  T  V  L  Q  Q  L  G  W  M  H  H  N  M  D  L  V  G  D  K  A  T  K  E  S  Y  A  I  Q  Y  L

811  CAAAAGTCTTTGGAGGCAGATCCTAATTCTGGCCAATCGTGGTATTTTCTTGGAAGGTGTTATTCAAGTATTGGGAAAGTTCAGGATGCC
271  Q  K  S  L  E  A  D  P  N  S  G  Q  S  W  Y  F  L  G  R  C  Y  S  S  I  G  K  V  Q  D  A

901  TTTATATCTTACAGGCAATCTATTGATAAATCAGAAGCAAGTGCAGATACATGGTGTTCAATAGGTGTGTTGTATCAGCAGCAAAATCAG
301  F  I  S  Y  R  Q  S  I  D  K  S  E  A  S  A  D  T  W  C  S  I  G  V  L  Y  Q  Q  Q  N  Q

991  CCTATGGATGCTTTACAGGCATATATTTGTGCTGTACAATTGGACCATGGGCATGCCGCAGCCTGGATGGACCTAGGTACTCTCTATGAA
331  P  M  D  A  L  Q  A  Y  I  C  A  V  Q  L  D  H  G  H  A  A  A  W  M  D  L  G  T  L  Y  E

1081  TCCTGCAATCAACCTCAAGATGCCATTAAATGCTACCTAAATGCAGCTAGAAGCAAACGTTGTAGTAATACCTCTACGCTTGCTGCAAGA
361  S  C  N  Q  P  Q  D  A  I  K  C  Y  L  N  A  A  R  S  K  R  C  S  N  T  S  T  L  A  A  R

1171  ATTAAATTTCTACAGAATGGTTCTGATAACTGGAATGGTGGCCAGAGTCTTTCACATCATCCAGTACAGCAAGTTTATTCGTTGTGTTTG
391  I  K  F  L  Q  N  G  S  D  N  W  N  G  G  Q  S  L  S  H  H  P  V  Q  Q  V  Y  S  L  C  L

1261  ACACCACAGAAATTACAGCACTTGGAACAACTGCGAGCAAATAGAGATAATTTAAATCCAGCACAGAAGCATCAGCTGGAACAGTTAGAA
421  T  P  Q  K  L  Q  H  L  E  Q  L  R  A  N  R  D  N  L  N  P  A  Q  K  H  Q  L  E  Q  L  E

1351  AGTCAGTTTGTCTTAATGCAGCAAATGAGACACAAAGAAGTTGCTCAGGTACGAACTACTGGAATTCATAACGGGGCCATAACTGATTCA
451  S  Q  F  V  L  M  Q  Q  M  R  H  K  E  V  A  Q  V  R  T  T  G  I  H  N  G  A  I  T  D  S

1441  TCACTGCCTACAAACTCTGTCTCTAATCGACAACCACATGGTGCTCTGACCAGAGTATCTAGCGTCTCTCAGCCTGGAGTTCGCCCTGCT
481  S  L  P  T  N  S  V  S  N  R  Q  P  H  G  A  L  T  R  V  S  S  V  S  Q  P  G  V  R  P  A

1531  TGTGTTGAAAAACTTTTGTCCAGTGGAGCTTTTTCTGCAGGCTGTATTCCTTGTGGCACATCAAAAATTCTAGGAAGTACAGACACTATC
511  C  V  E  K  L  L  S  S  G  A  F  S  A  G  C  I  P  C  G  T  S  K  I  L  G  S  T  D  T  I
```

FIG. 4B

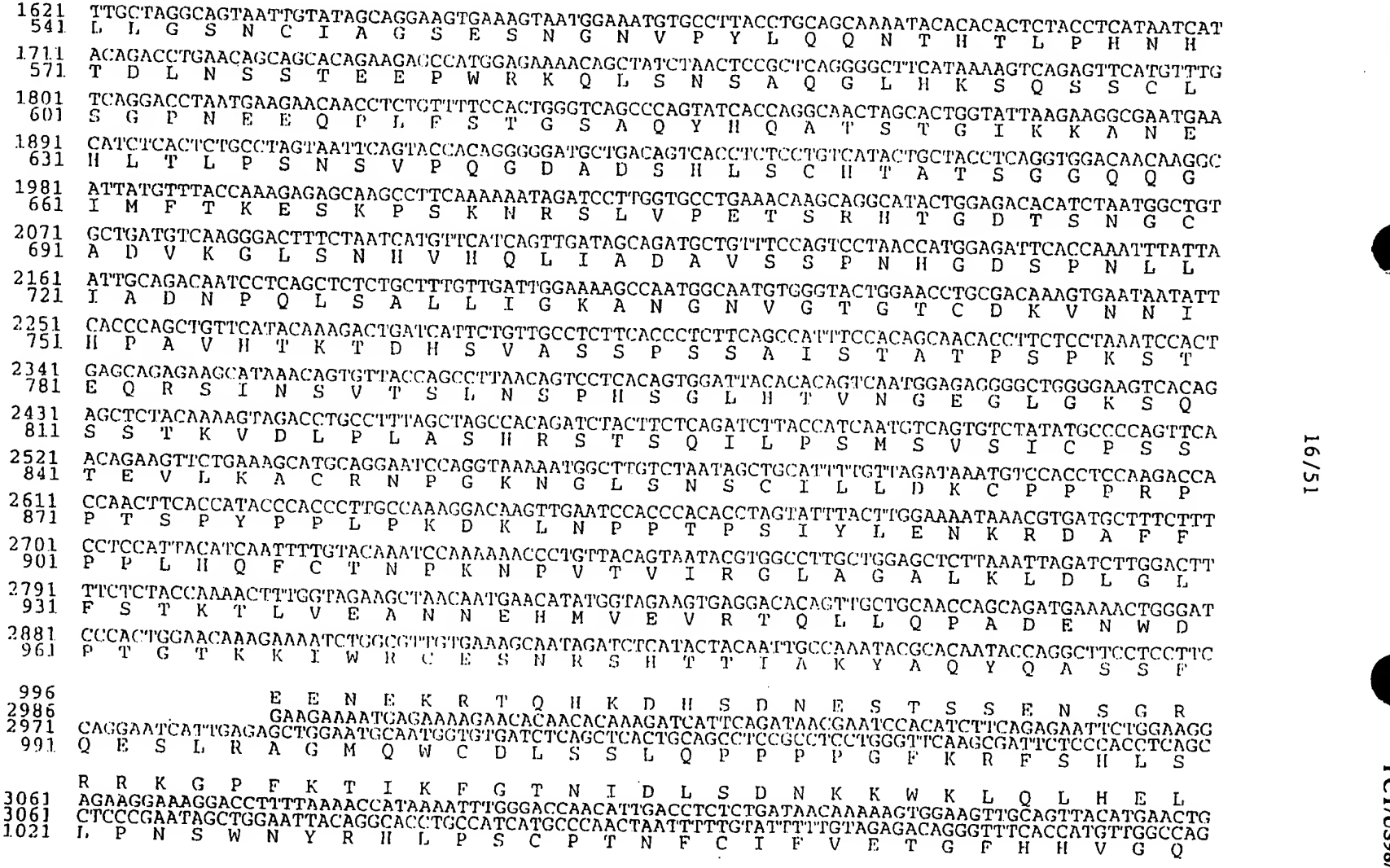

FIG. 4C

```
                1051  T  K  L  P  A  F  A  R  V  V  S  A  G  N  L  L  T  H  V  G  H  T  I  L  G  M  N  T  V  Q
Medium          3151  ACTAAACTTCCTGCTTTTGCGCGTGTGGTGTCAGCAGGAAATCTTCTAACCCATGTTGGGCATACCATTCTGGGCATGAATACAGTACAA
Short           3151  GCTTGTCTTGAACTCCTGACCTCAGGTGGTCTGCTTGCCTCAGCATCCCAAAGTGCTGGGATTACAGGTGTGAGCCACCATGCCCGGTAA
                1051  A  C  L  E  L  L  T  S  G  G  L  L  A  S  A  S  Q  S  A  G  I  T  G  V  S  H  H  A  R  *    1079

                1081  L  Y  M  K  V  P  G  S  R  T  P  G  H  Q  E  N  N  N  F  C  S  V  N  I  N  I  G  P  G  D
Medium          3241  CTGTATATGAAAGTTCCAGGGAGTCGGACACCAGGTCACCAAGAAAATAACAACTTCTGCTCTGTTAACATAAATATTGGTCCAGGAGAT
Short           3241  acttttaaaaatgtaagcaaaattacagtatgtaaaacacacattgctaatggagaaataaagttcctacttttacatctaaaaaaaaaa  3330

                1111  C  E  W  F  V  V  P  E  D  Y  W  G  V  L  N  D  F  C  E  K  N  N  L  N  F  L  M  S  S  W
Medium          3331  TGTGAATGGTTTGTTGTACCTGAAGATTATTGGGGTGTTCTGAATGACTTCTGTGAAAAAAATAATTTGAATTTTTTAATGAGTTCTTGG

                1141  W  P  N  L  E  D  L  Y  E  A  N  V  P  V  Y  R  F  I  Q  R  P  G  D  L  V  W  I  N  A  G
Medium          3421  TGGCCCAACCTTGAAGATCTTTATGAAGCAAATGTCCCTGTGTATAGATTTATTCAGCGACCTGGAGATTTGGTCTGGATAAATGCAGGC

                1171  T  V  H  W  V  Q  T  V  G  W  C  N  N  I  A  W  N  V  G  P  L  T  A  C  Q  Y  K  L  A  V
Medium          3511  ACTGTGCATTGGGTTCAAACTGTTGGCTGGTGCAATAACATTGCCTGGAATGTTGGTCCACTTACAGCCTGCCAGTATAAATTGGCAGTG

                1201  E  R  Y  E  W  N  K  L  K  S  V  K  S  P  V  P  M  V  H  L  S  W  N  M  A  R  N  I  K  V
Medium          3601  GAACGGTATGAATGGAACAAATTGAAAAGTGTGAAGTCACCAGTACCCATGGTGCATCTTTCCTGGAATATGGCACGAAATATCAAAGTC
```

FIG. 4D

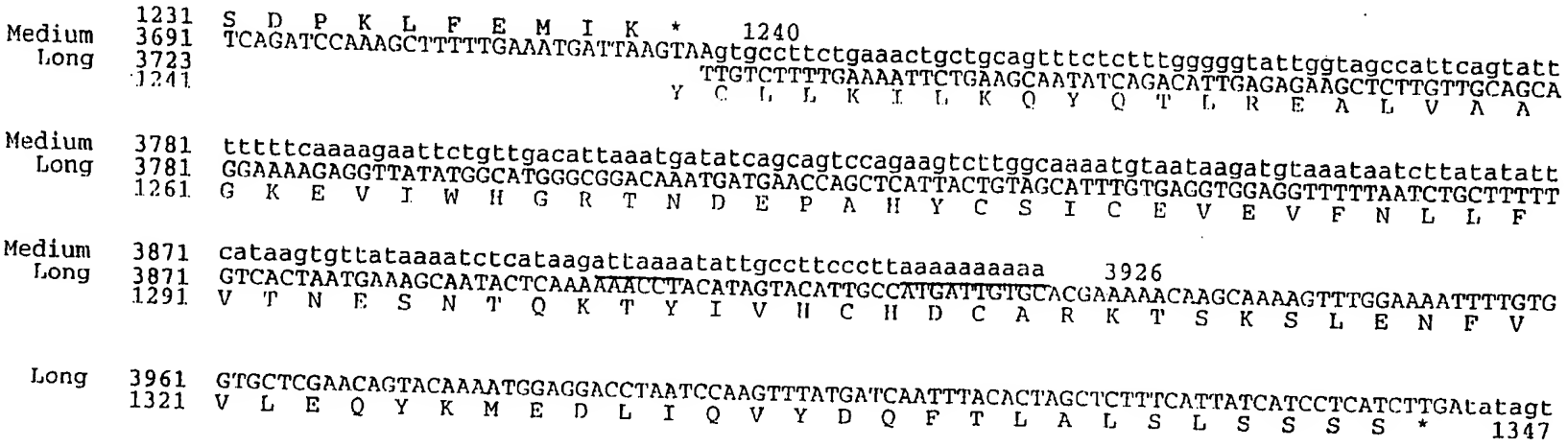

FIG. 4E

```
Long  4051  tccatgaatattaaatgagattatttctgctcttcaggaaatttcctgcaccactggttttgtagctgtttcataaaaactgttgactaaaa
Long  4141  gctatgtctatgcaaccttccaagaatagtatgtcaagcaactggacacagtgctgccttctgcttcaggacttaacatgctgatccagct
Long  4231  gtacttcagaaaaaataattaatcatatgttttgtgtacgtatgacaaagtgacacagaatactgatttgaagatagccttt
Long  4321  tttatgtttctctatttctgggctgatgaattaatattcatttgtatttaaccctgcagaatttttccttagttaaaaacactttccta
Long  4411  gctggtcatttcttcataagatagcaaatttaaatctcctcgatcagctttaaaaaatgtgtactatattatcctgaggaagtttttttac
Long  4501  tgctttatgttttgtgtttgaggccatgatgattacatttgtggttccaaaataatttttttaaatattaatagcccatatacaaa
Long  4591  gataatggattgcacatagacaaagaaataaacttcagatttgtgatttttgttctaaacttgatacagatttacactatttataaata
Long  4681  cgtatttattgcctgaaaatatttgtgaatggaatgttgttttttccagacgtaactgccattaaatactaaggagttctgtagtttta
Long  4771  aacactactcctatttacatttatatgtgtagataaaaactgcttagtattatacagaaatttttattaaaattgttaaatgttaaaggg
Long  4861  tttcccaatgtttgagtttaaaaaagacttttctgaaaaaatccacttttgttcatttttcaaacctaatgattatatgtatttttatatgt
Long  4951  gtgtgtatgtgtacacacatgtataatatatacagaaacctcgatatatataattgtatagatttaaaagtttttatttttacatctatgg
Long  5041  tagttttgaggtgcctattataaagtattacggaagtttgctgttttaaagtaaatgtcttttagtgtgatttattaagttgtagtca
Long  5131  ccatagtgatagcccataaataattgctggaaaattgtatttttataacagtagaaaacatatagtcagtgaagtaaatatttttaaaggaa
Long  5221  acattatatagatttgataaatgttgtttataattaagagtttcttatggaaaagagattccagaatgataacctcttttagagaacaaat
Long  5311  aagtgacttatttttttaaagctagatgactttgaaatgctatactgtcctgcttgtacaacatggtttgggtgaaggggaggaaagta
Long  5401  ttaaaaaatctatatcgctagtaaattgtaataagttcttattcatatgaaaaaaaaaa                    5471
```

FIG. 4F

TB4X & TB4Y

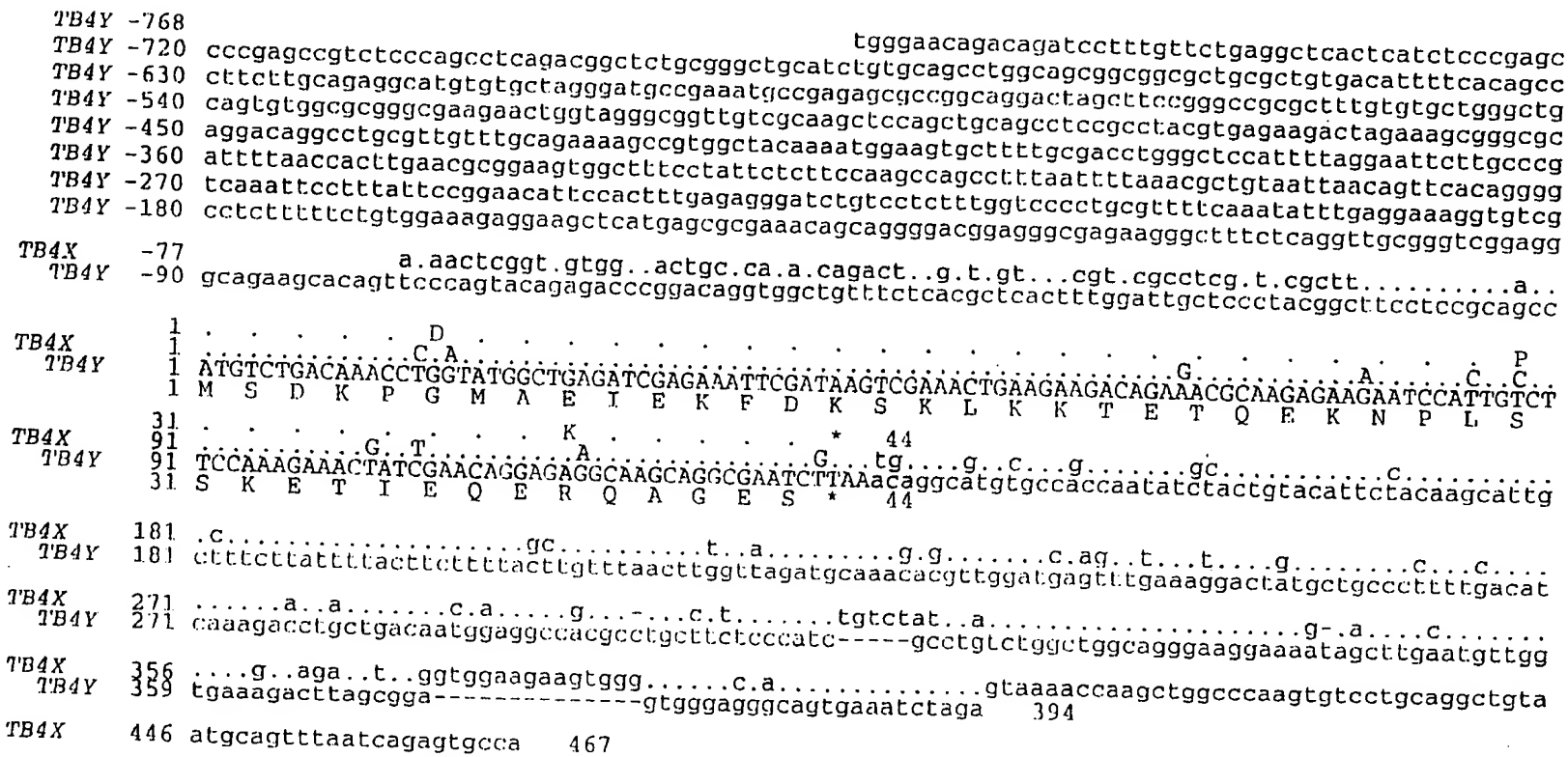

FIG. 5

EIF1AX & EIF1AY

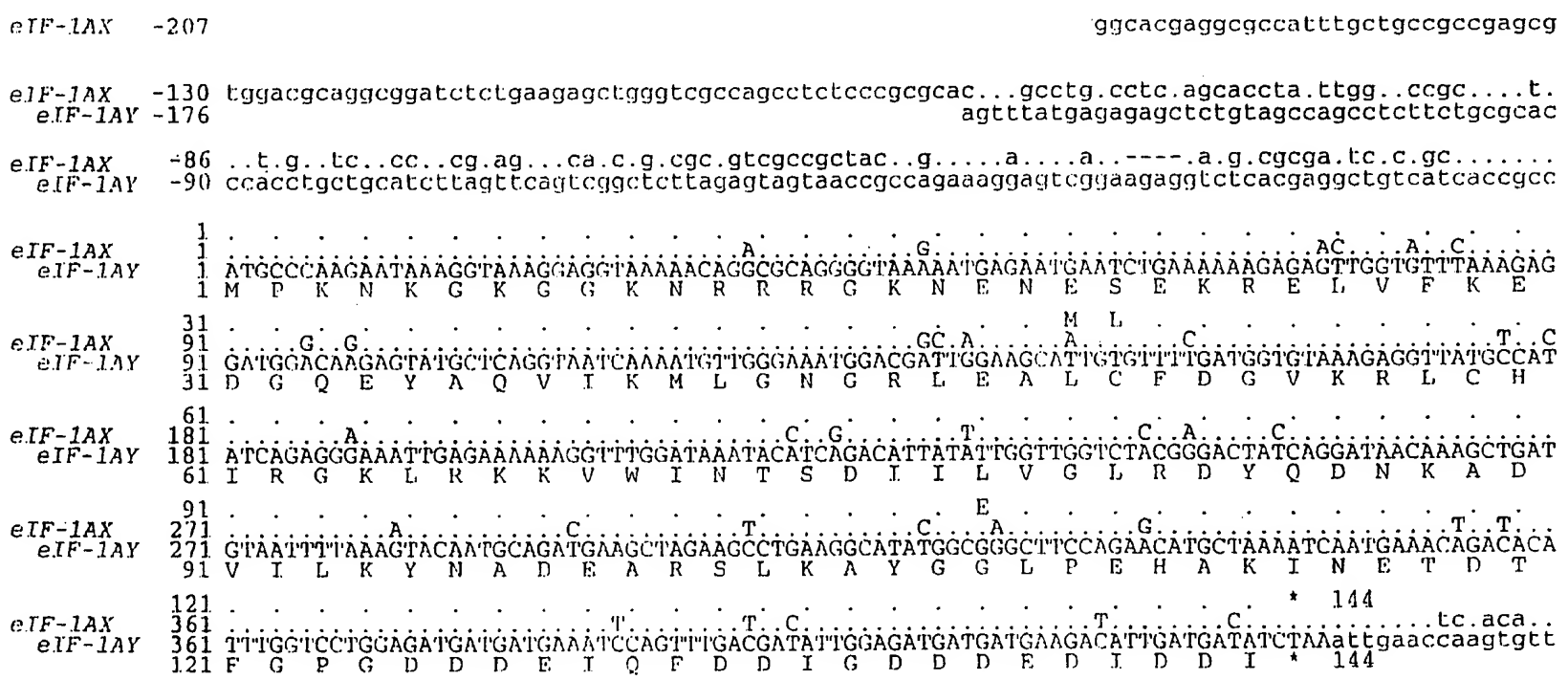

FIG. 6A

```
eIF-1AX   451 .....tc..tc..t.....a...t..c.....a..t.........at...g.-...ag....tt.a..t......agcat.aa..c.a.
eIF-1AY   451 tttacatgacaagttctctgaggatggttctacagttgggatttttggccatcatcaaccaagaagagaaattcatttta---gtgtgtagt

eIF-1AX   539 ..g.t......g........g...ct.aga.a..-------...gg..t...t...a....ta.t.atgc.......t.....g.g.g...
eIF-1AY   538 ttctgaaagcaaactgatttatttttcattgttttaaagtatttatttctttaaaagctgaggaca---ttgaattaccttaatttaaatg

eIF-1AX   623 ....gccc.....g..c....a.........g....t..g..g.......gtctac.aa.tac....a...a...aa.ccatga..g.
eIF-1AY   625 ttaa---tactttattgttttgatgtaatggaacttaaggataaaagaccataatatttgctgttaaaataaataaacgagtgcctttcc

eIF-1AX   713 .g..t.tcct..ca.ttccagt...a..a...g.g.......g.a..cat.tg.c--.tg..tca.g..a.ctg.t........ggcct.
eIF-1AY   712 tactgtgataacgtc-------aagtaattggatattttgaatacatttctgcctgataatcatgctgagttctaataagccctacttcc

eIF-1AX   801 ------.tga.g..t..ag.ca...-..a..c.......c.....at..c.t.g..a...-..............ggg..g.....g....
eIF-1AY   795 acctaatctgtttacagtcttttggtatgtttcagttacttagatggtctcataaggtttctgatacaatttga---agacagaaatctg

eIF-1AX   883 ..g..ga.g--.a....a..aagtc.g...g.........ag...c.tg.gc...t......a..g.tt......tca.g.tct.ttca.
eIF-1AY   882 catttagaatcagaaaacatggacatatttttcatatttatctagtc-atatgtaatttttatgctaacattgatagtttataaatccttt

eIF-1AX   971 agtcgta..t....a.c..a.ac.t
eIF-1AY   971 tcatcctttgtgcctcggttattaaggaaaaaaaaatgtccaacatacagtttttaaagtgtggcagttttgagtagtaacttagaatgt

eIF-1AY 1061 ataagattaagagttaaagaaaccgaacaataagtggcaaccaattatcttaacattggaaatactggggtgccatttgttttcaaaag
eIF-1AY 1151 ttattcattgtaatccactgttttggctttcatgaacaagtaaattacagtgtataaatgaaaagcaatttcataataaattctataaac
eIF-1AY 1241 tgaaaaaaaaaa     1252
```

FIG. 6B

DFFRX & DFFRY

```
DFFRY  -1664                                                         gaagtgacatgttggcatgggcccaattctgctggtcctttagt
DFFRY  -1620  atacaaaaaaaataaaggtttaccagtatgtcactacatgcagatttatggattgtacagaaaattggtgattcccaaatttcactgtgc
DFFRY  -1530  atcaaaataatcgatggaactttaaagactaaagatttctagaccccaccccaggcccgatgattgagaatatctagaggggacccaaga
DFFRY  -1440  atccatatatttaagtgccccacccacaacaatgacctttaagcaggtagtttgcatttgggaaccactgctacaggttactagtgggac
DFFRY  -1350  aaccagttaggagcataagtttgaacattttacagtttgtcacctgtgatagcttatcacctgtgatataaccagaaatccaattaagat
DFFRY  -1260  tgctatctctctgtaatctgtttgcaatttaggtgttaatttttttgaaagttcagaaaaaagtagacaaaacagaaaagaaatcaagta
DFFRY  -1170  caactacataatgacaaaaaacgtattacacttgtattaaacttcaaaactggagaataaaggtgcaatataacatgaaaataattaaat
DFFRY  -1080  gctaagtgaaataatatcaaatgtagttgaccctgaagaaaatgcagtagtgagggatccctaacctgtgggccctccaggaattactgt
DFFRY   -990  tgaatggtcttgagaatccactggaaaagaccaagcattgttacctgaataattgaactttgtttatttctccatattttgcagtggta
DFFRY   -900  attccattataaaacctaatgaaacaatgtttttatagatggtgtggaaagactttttctgggctcagaggtgaaactgacccttgtgtat
DFFRY   -810  cagcagcatttctgactgactgagagagtgtagtgattaacagagttgtgatgttagttaagaaacttagatttgccattgtagcttttc
DFFRY   -720  taccaattagcagattgtttaactcactgaaattgtaaagtggtagacgtggacttagtcattactgggcagcttatgaattgtattcat
DFFRY   -630  ttactcatgatgtaaaaatggttagtctccactttttaaggctctagttctagtggctaaataggtacttatttatacagtatgataactg
DFFRY   -540  ctgtattaaaatacatgtctcaaatgtggaatagtagaagaggtgaagaaaatcatagtttgaggtagaatactgtttgctggtcttaaa
DFFRY   -450  aactgtggtatttggtgattccataaattaggtcagatacttccactggagggaaacagtttaaaggatatatgtgatactattaatag
DFFRY   -360  aatgaggaagacacaccagatatttaggagggaattagcgagcttgaaactaagagctggtttgaatgagactgggtcataagtgatttc
DFFRY   -270  aagtaccagattaaggcactgagattttatttttaagcactgaagtcagattttttcctttaaaagaaaggattcatgatgaaatctgc
DFFRY   -180  tttttgttttgcagagagcttggagataattctggtggctgtgtggagtatgtgttggaggtattaaattttcacagtatatataaggca

DFFRX    -59                                       c.tttct..ag.ca.ctac.t..gc...c....tt..........cc.......g...
DFFRY    -90  gcaattgataggcctttcacagatttcttctgataactacataaagagacaaaaaaaagaaaaaagagcaaagatctgtgctgtgtcaagt
```

FIG. 7A

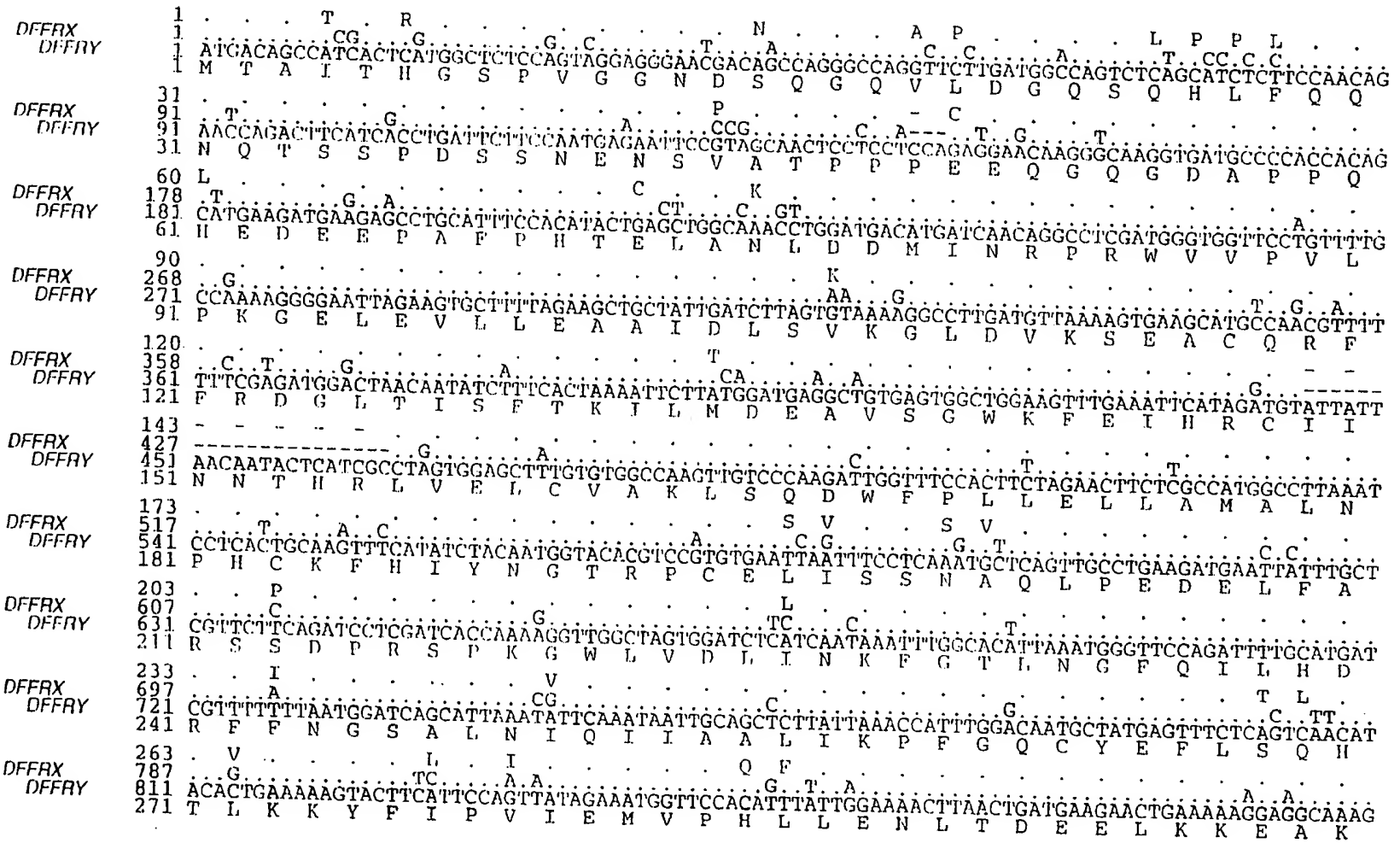

FIG. 7B

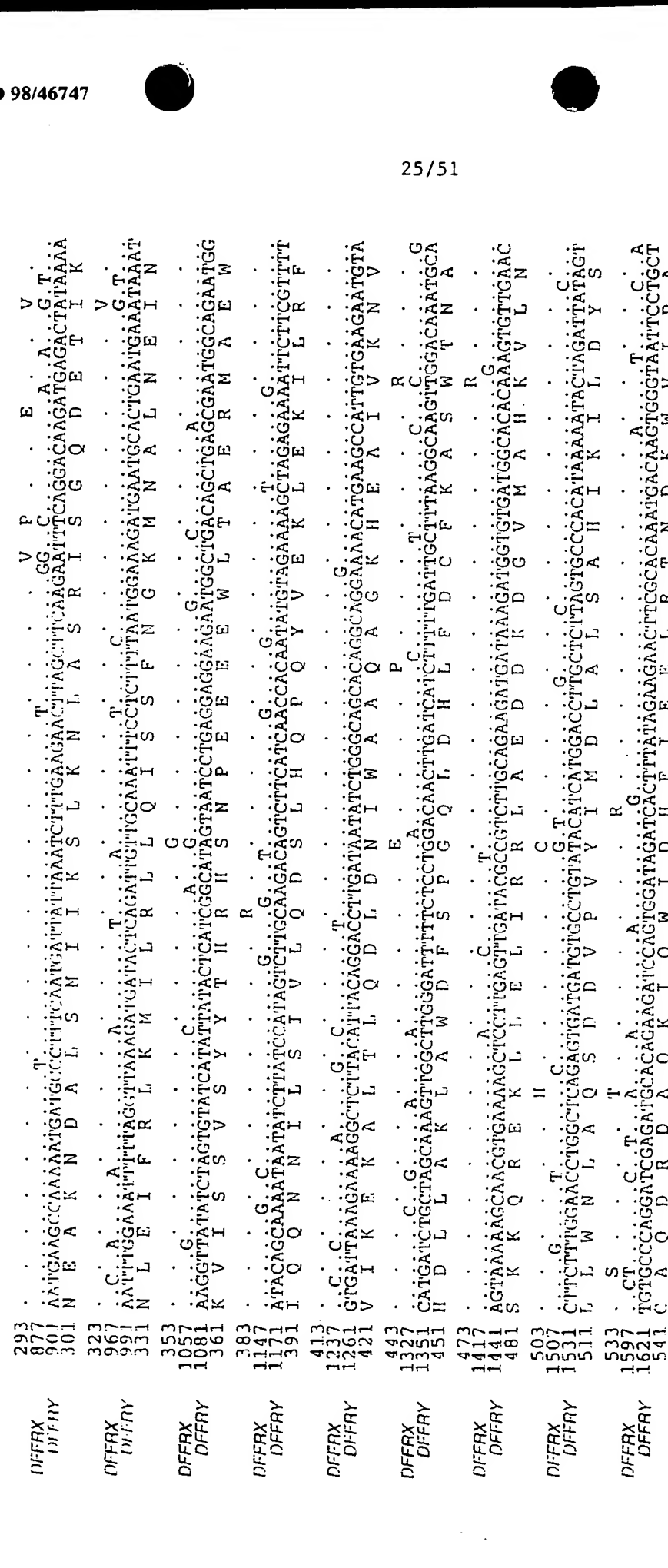

FIG. 7C

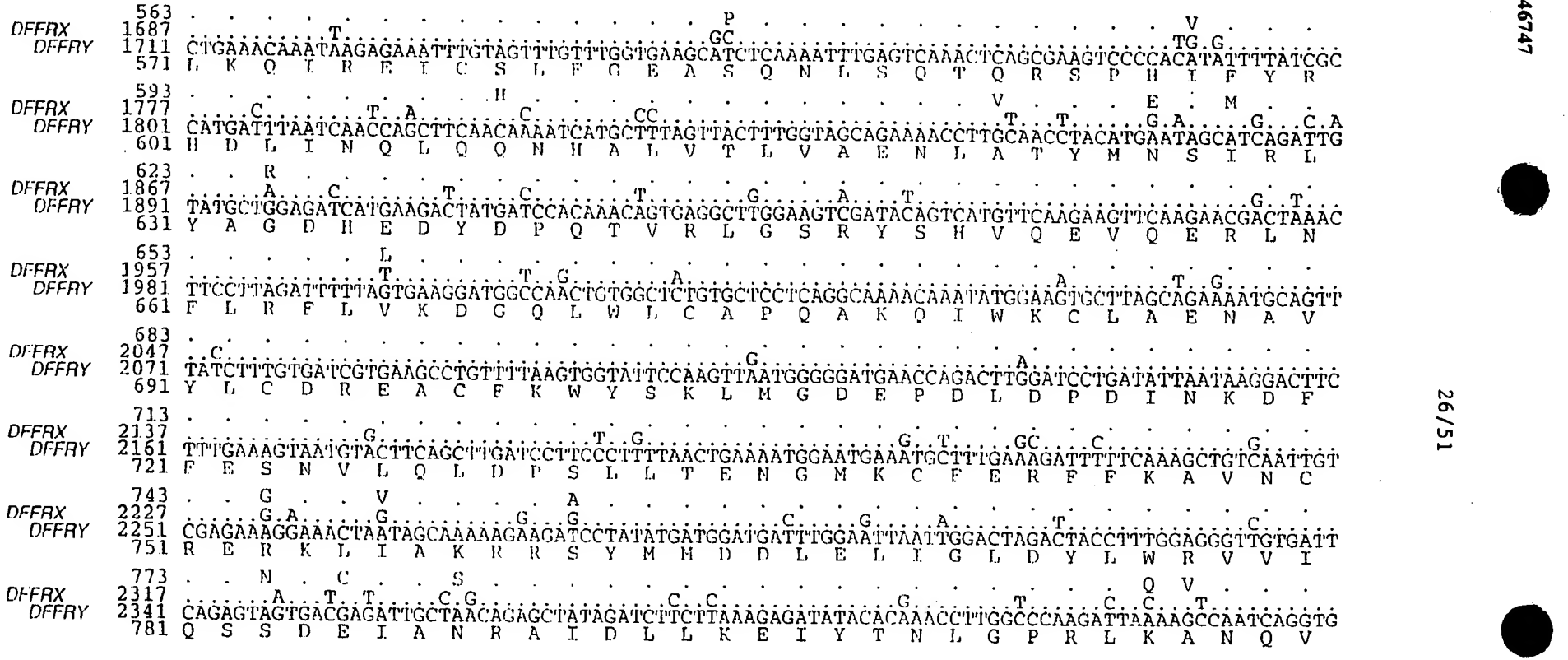

FIG. 7D

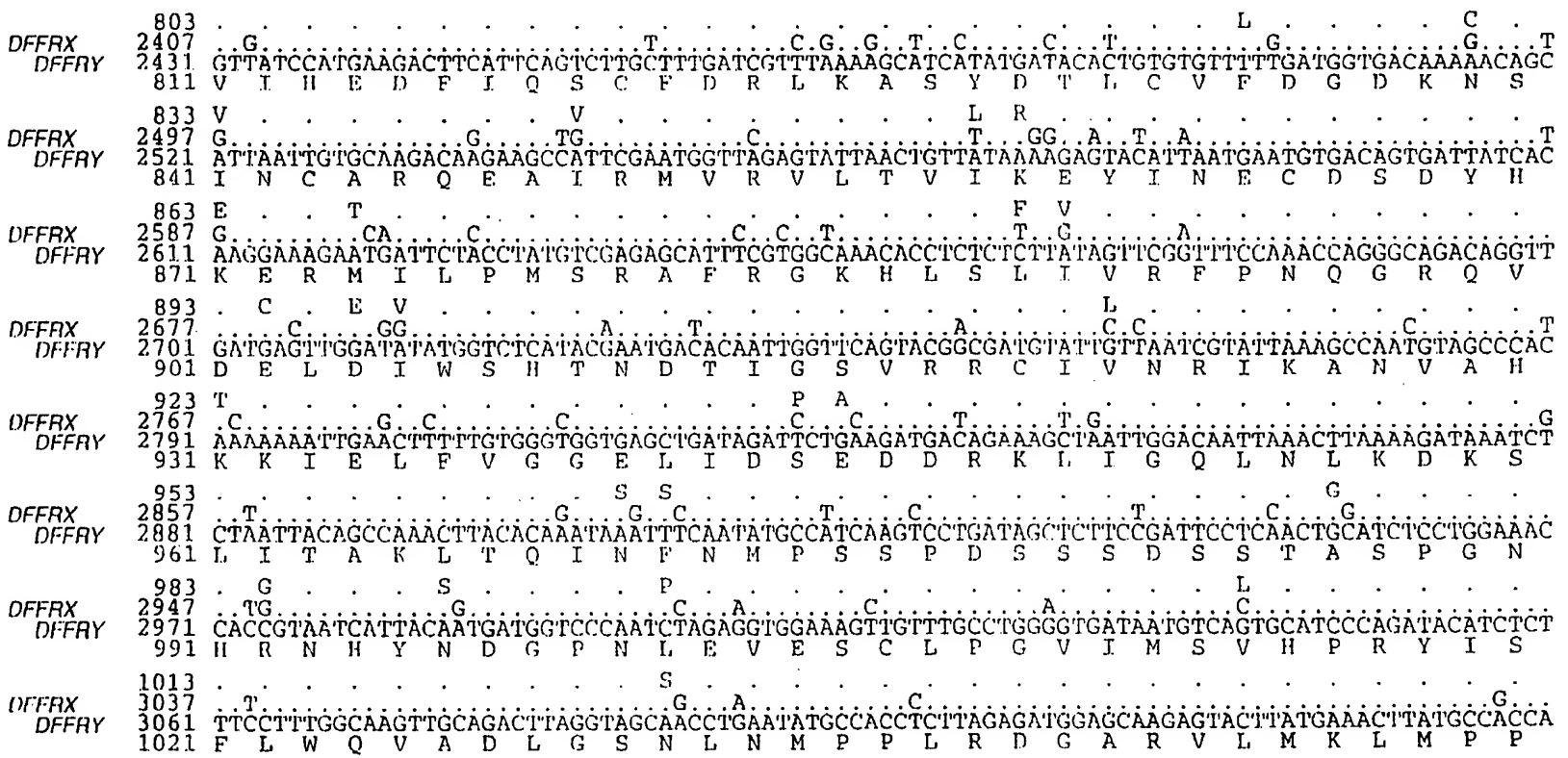

FIG. 7E

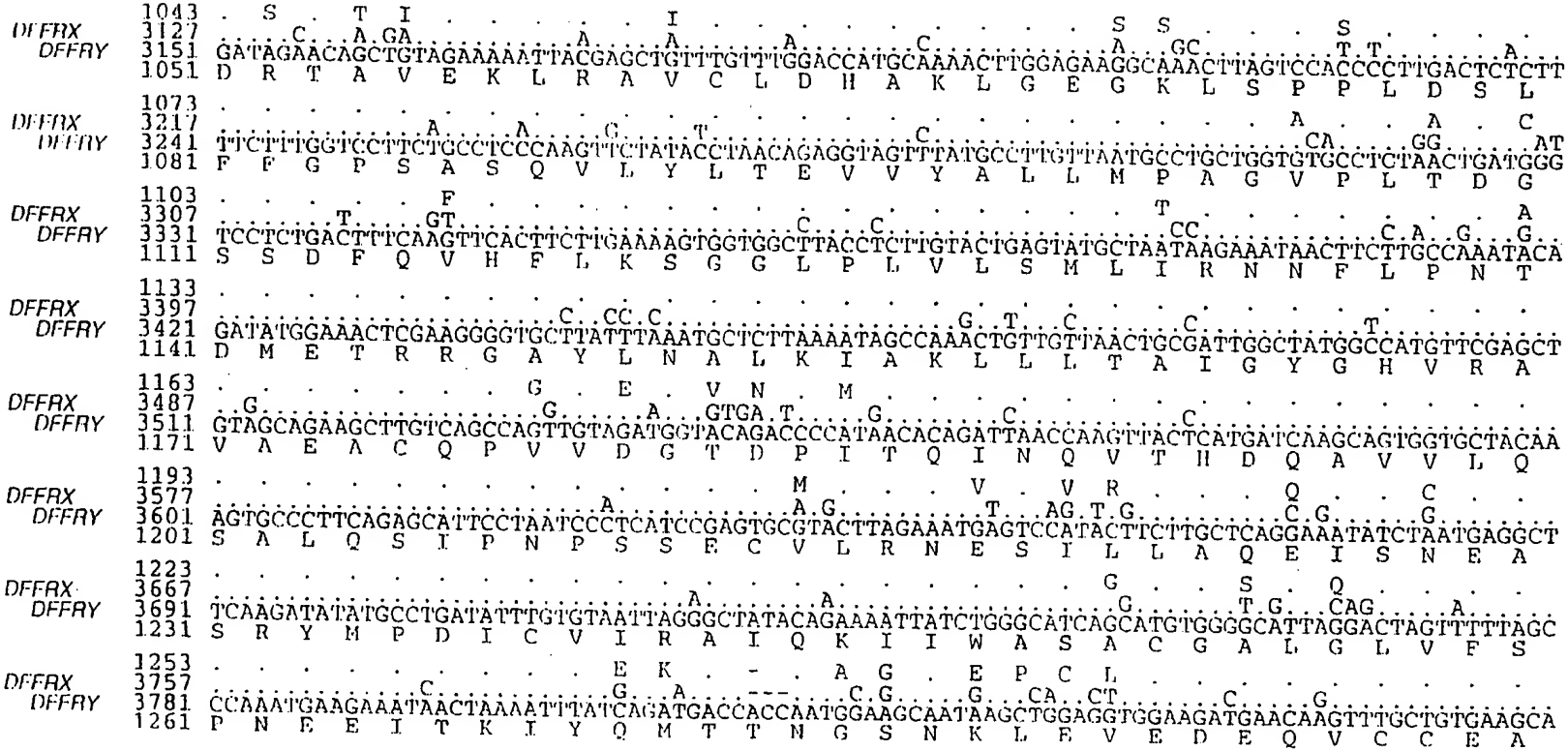

FIG. 7F

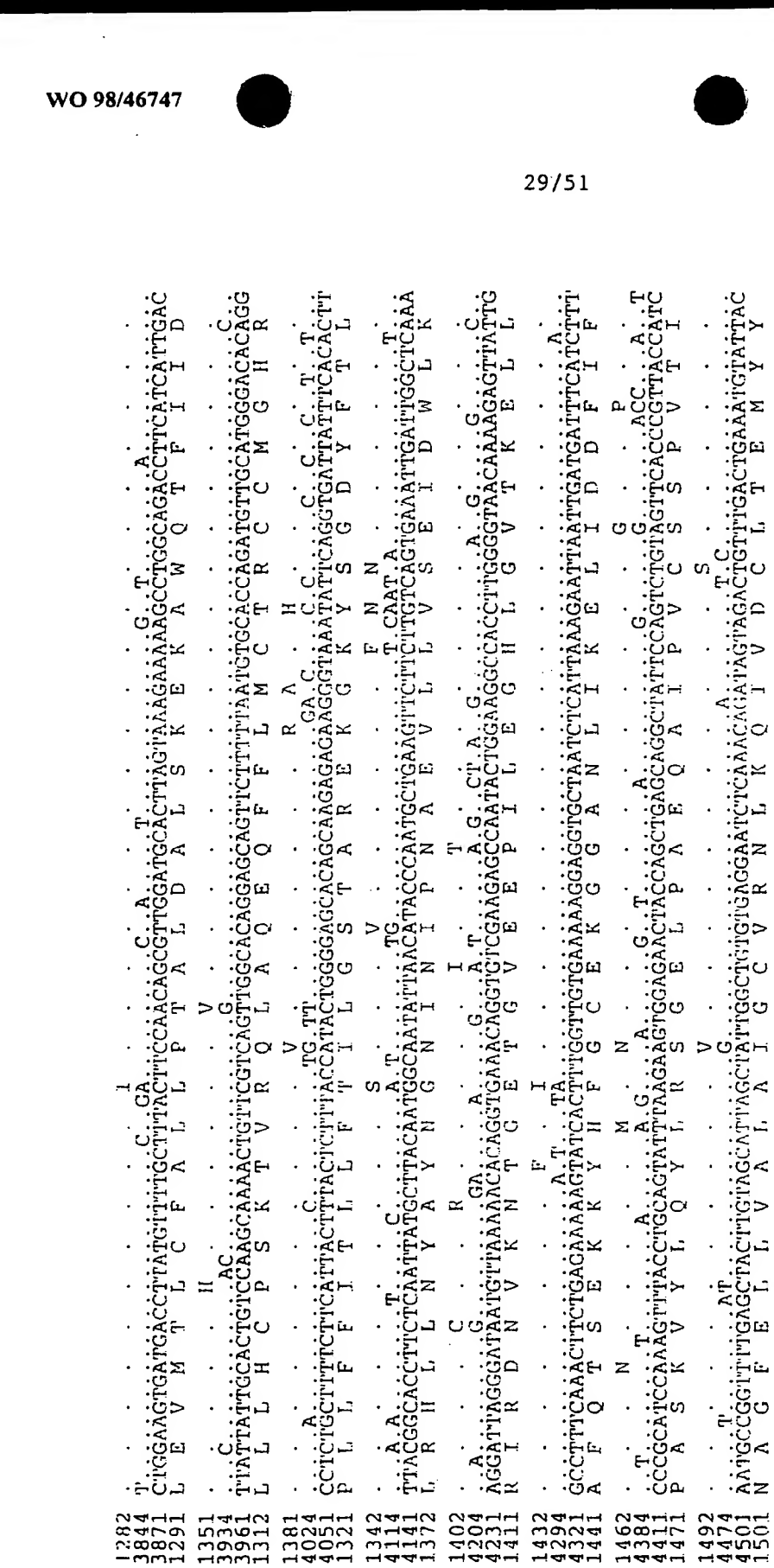

FIG. 7G

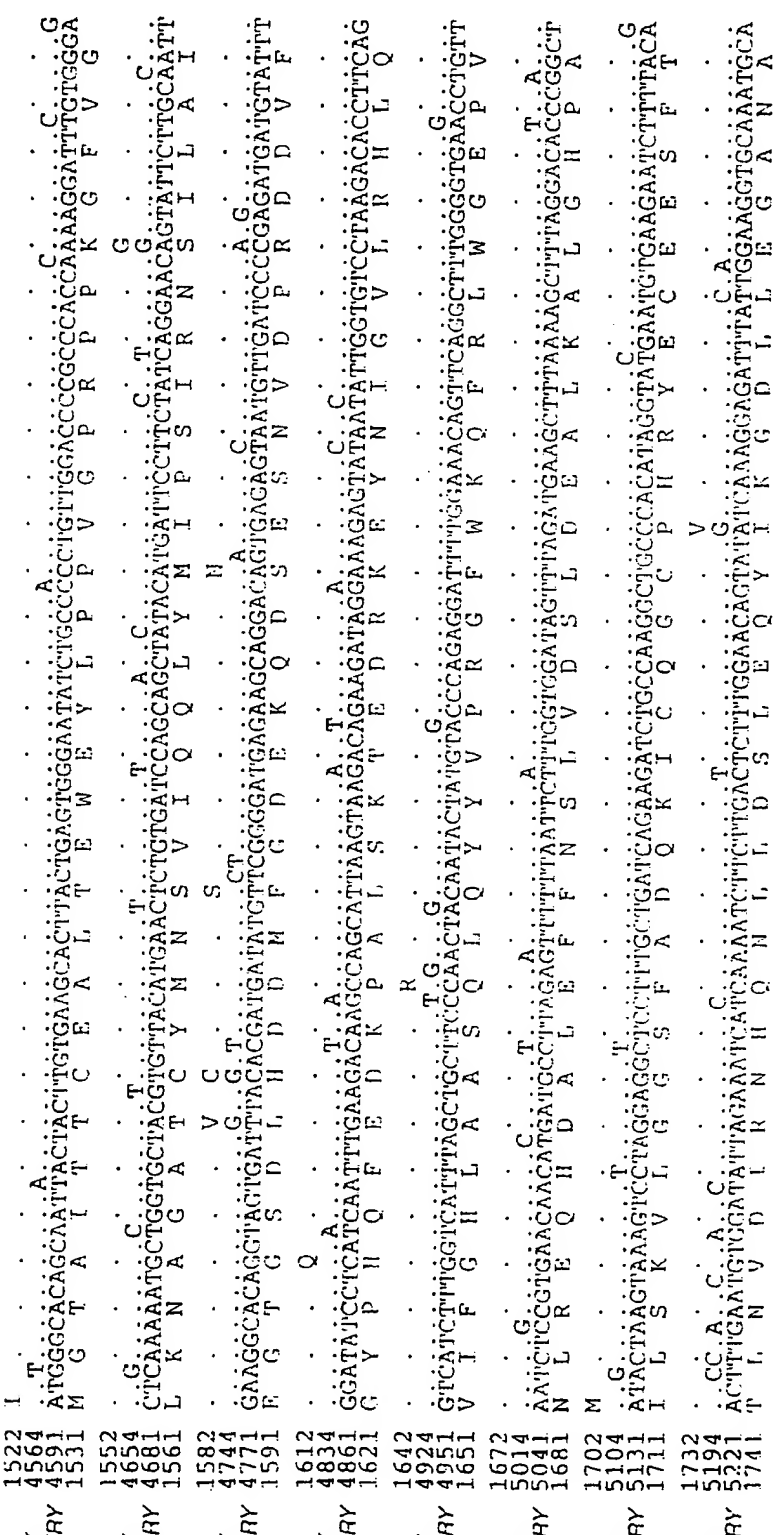

FIG. 7H

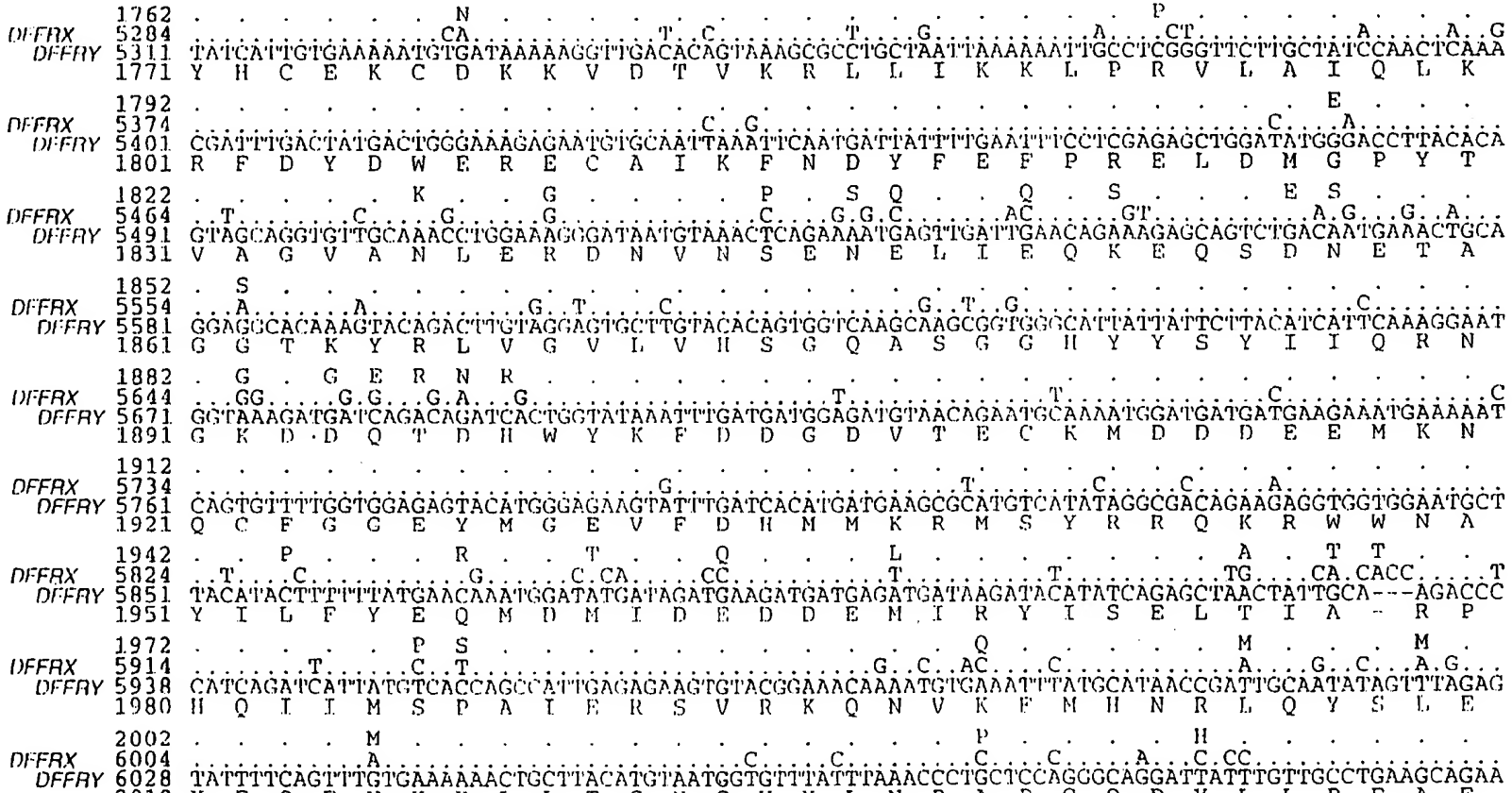

FIG. 7I

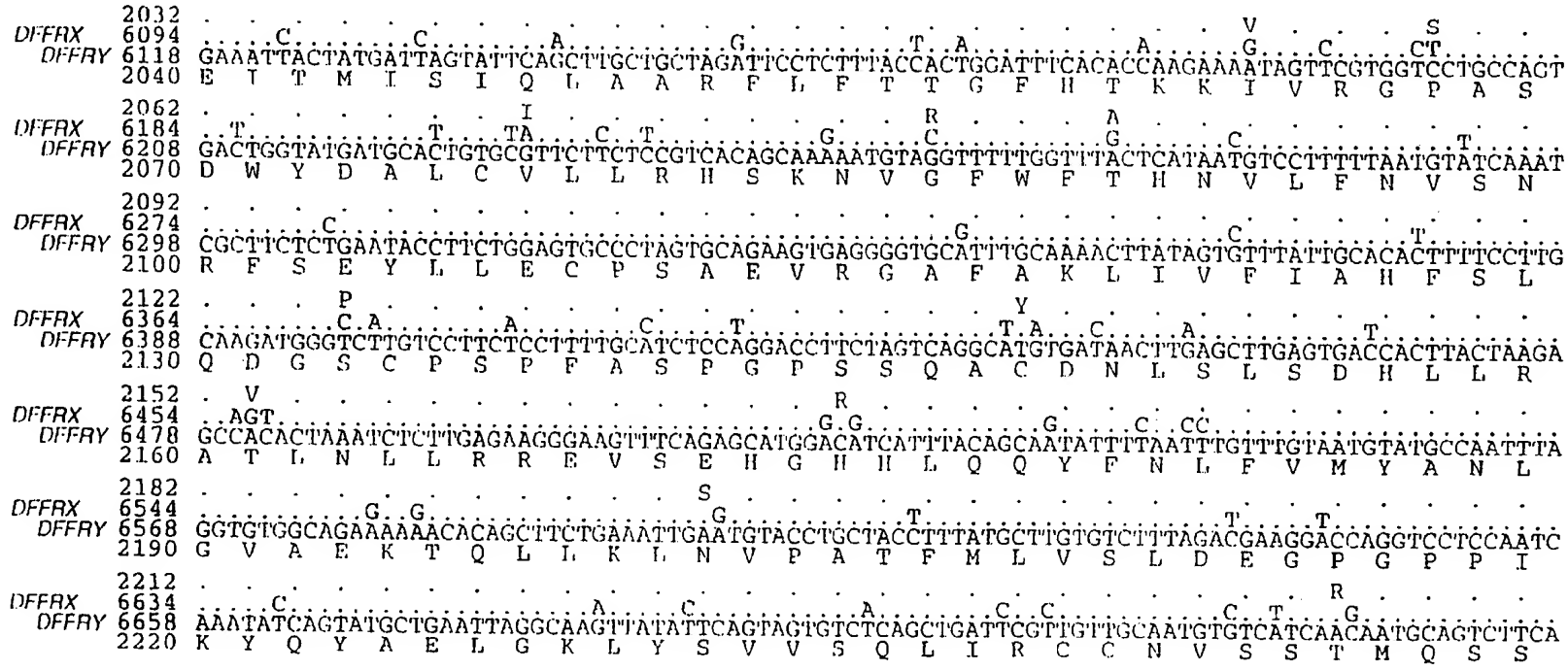

FIG. 7J

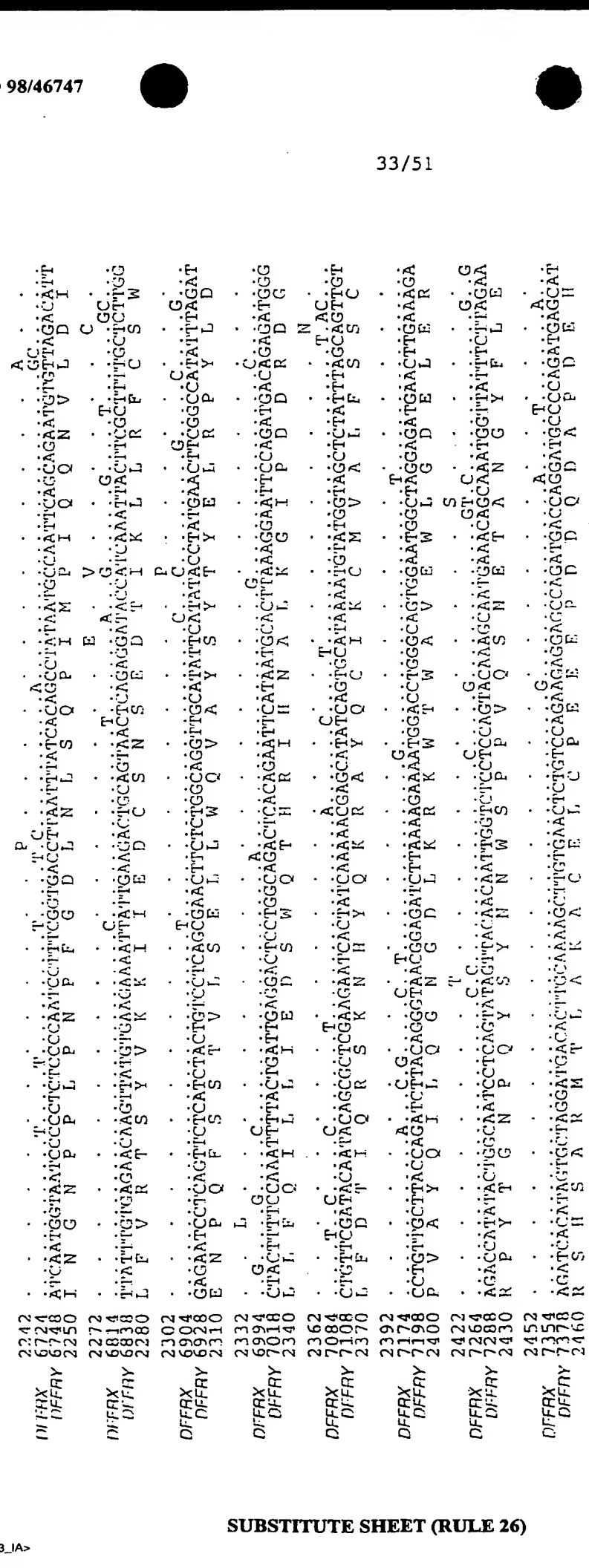

FIG. 7K

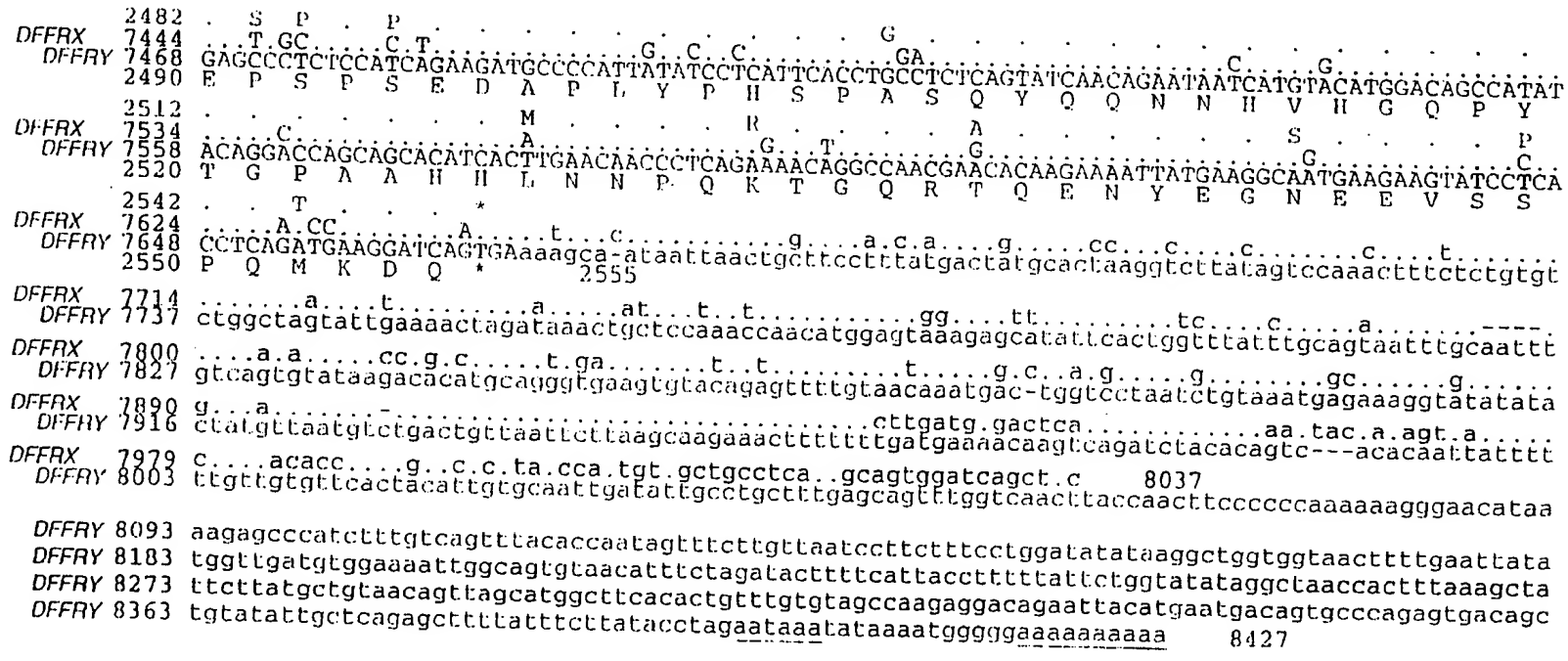

FIG. 7L

CDYa & CDYb

```
CDYa  -281                                                                                ctgtggattta
CDYb  -328  gtaacaggcaggaagaaagctttctgtactacaccagagggttgggg...........

CDYa  -270  gctactctcacctgaggctactgagcaagttgtcatgcaccatgagacaaagcccaagctgtcccaccaggcagtaagtatggagaggtt
CDYb  -270  .............................c..................................................g.........

CDYa  -180  caggcacatggcatagctgctatttcgcacaattttcactacaccagtggtgacaaaatagaagaggttcatccatacacagaacctggt
CDYb  -180  ......................................................c...................................

CDYa   -90  gaagagctggaggcagaaagaagtgtctatgtggagacgcaactgaaacaaaggtggcacagcaactgttccaatcccgtgtctttcctc
CDYb   -90  ..........................................................................................

         1   M  A  S  Q  E  F  E  V  E  A  I  V  D  K  R  Q  D  K  N  G  N  T  Q  Y  L  V  R  W  K  G
CDYa     1  ATGGCTTCCCAGGAGTTTGAGGTTGAAGCTATTGTTGACAAAAGACAGGATAAAAATGGGAATACACAGTATTTGGTTCGGTGGAAAGGT
CDYb     1  ..........................................................................................
         1   .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .

        31   Y  D  K  Q  D  D  T  W  E  P  E  Q  H  L  M  N  C  E  K  C  V  H  D  F  N  R  R  Q  T  E
CDYa    91  TATGACAAACAGGATGACACTTGGGAACCAGAGCAGCACCTCATGAACTGTGAAAAATGTGTACATGATTTTAATAGACGACAGACTGAA
CDYb    91  ...................................................A......................................
        31   .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  K  .  .  .  .  .  .  .  .  .  .  .  .

        61   K  Q  K  K  L  T  W  T  T  T  S  R  I  F  S  N  N  A  R  R  R  T  S  R  S  T  K  A  N  Y
CDYa   181  AAACAGAAAAAACTGACATGGACTACAACCAGTAGAATTTTTTCAAACAATGCCAGAAGAAGAACTTCCAGATCTACAAAAGCAAACTAT
CDYb   181  ..........................................................................................
        61   .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .

        91   S  K  N  S  P  K  T  P  V  T  D  K  H  H  R  S  K  N  R  K  L  F  A  A  S  K  N  V  R  R
CDYa   271  TCTAAGAACTCTCCTAAAACGCCAGTGACTGATAAACACCACAGGTCCAAAAACCGCAAGTTATTTGCTGCCAGCAAGAACGTTAGGAGA
CDYb   271  ....................T.................................T...................................
        91   .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  C  .  .  .  .  .  .  .  .  .  .  .

       121   K  A  A  S  I  L  S  D  T  K  N  M  E  I  I  N  S  T  I  E  T  L  A  P  D  S  P  F  D  H
CDYa   361  AAGGCAGCTTCAATTCTCTCCGACACAAAGAATATGGAGATAATAAATTCAACTATTGAGACCCTTGCACCTGACAGCCCCTTTGACCAC
CDYb   361  .............C............................................................................
       121   .  .  .  .  T  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .

       151   K  -  T  V  S  G  F  Q  K  L  E  K  L  N  P  I  A  A  D  Q  Q  D  T  V  V  F  K  V  T  E
CDYa   451  AAA---ACTGTGAGTGGCTTTCAGAAACTTGAGAAACTGAACCCTATTGCAGCAGATCAGCAGGACACGGTGGTCTTCAAGGTGACAGAA
CDYb   451  ...AAA.................................G..................................................
       151   .  K  .  .  .  .  .  .  .  .  .  .  .  D  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .

       180   G  K  L  L  R  D  P  L  S  R  P  G  A  E  Q  T  G  I  Q  N  K  T  Q  I  H  P  L  M  S  Q
CDYa   538  GGGAAACTCCTCCGGGACCCTTTGTCACGTCCTGGTGCAGAACAGACTGGAATACAGAACAAGACTCAGATACACCCACTAATGTCGCAG
CDYb   541  ............................A..........................................G..................
       181   .  .  .  .  .  .  .  .  .  H  .  .  .  .  .  .  .  .  .  .  .  .  .  M  .  .  .  .  .  .

       210   M  S  G  S  V  T  A  S  M  A  T  G  S  A  T  R  K  G  I  V  V  L  I  D  P  L  A  A  N  G
CDYa   628  ATGTCTGGCTCAGTTACTGCTTCTATGGCCACAGGTTCAGCTACCCGAAAGGGTATAGTGGTATTAATAGACCCATTAGCAGCCAATGGG
CDYb   631  ..........................................................................................
       211   .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
```

FIG. 8A

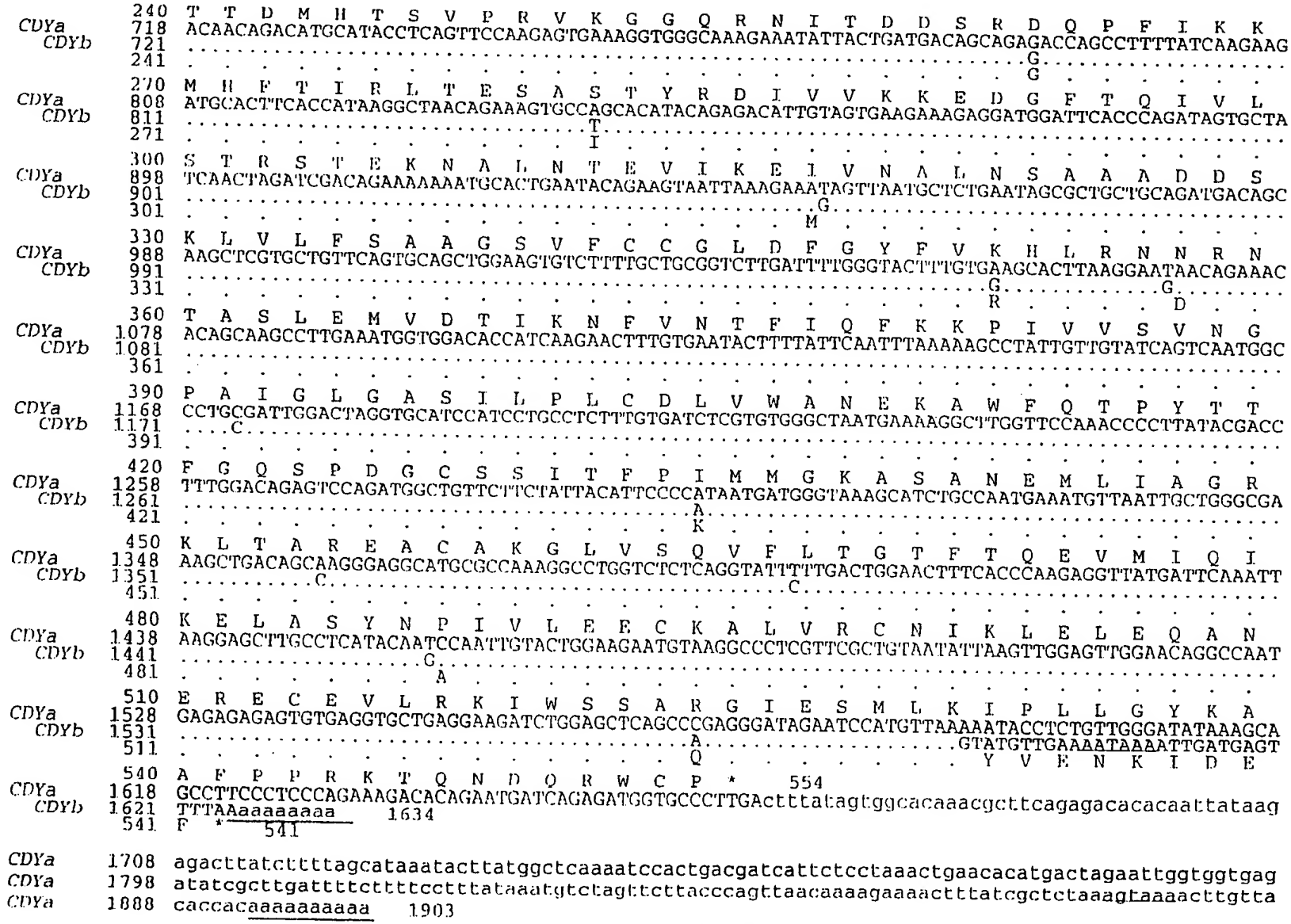

FIG. 8B

BPY1

```
-72                gagaggggtatacacagggaggccaggcagcctggagttagtcgaccgttgcgagacgttgagctgcggcag

  1  ATGAGTCCAAAGCCGAGAGCCTCGGGACCTCCGGCCAAGGCCAAGGAGACAGGAAAGAGGAAGTCCTCCTCTCAGCCGAGCCCCAGTGGC
  1  M  S  P  K  P  R  A  S  G  P  P  A  K  A  K  E  T  G  K  R  K  S  S  S  Q  P  S  P  S  G

 91  CCGAAGAAGAAGACTACCAAGGTGGCCGAGAAGGGAGAAGCAGTTCGTGGAGGGAGACGCGGGAAGAAAGGGGCTGCGACAAAGATGGCG
 31  P  K  K  K  T  T  K  V  A  E  K  G  E  A  V  R  G  G  R  R  G  K  K  G  A  A  T  K  M  A

181  GCCGTGACGGCACCTGAGGCGGAGAGCGGGCCAGCGGCACCCGGCCCCAGCGACCAGCCCAGCCACGAGCTCCCTCAGCACGAGCTGCCG
 61  A  V  T  A  P  E  A  E  S  G  P  A  A  P  G  P  S  D  Q  P  S  Q  E  L  P  Q  H  E  L  P

271  CCGGAGGAGCCAGTGAGCGAGGGGACCCAGCACGACCCCCTGAGTCAGGAGAGCGAGCTGGAGGAACCACTGAGTAAGGGGCGCCCATCT
 91  P  E  E  P  V  S  E  G  T  Q  H  D  P  L  S  Q  E  S  E  L  E  E  P  L  S  K  G  R  P  S

361  ACTCCCCTATCTCCCTGAgcagcaactaagtttaggcccagctgccagacctcagagatctcaccagcagggtgcttcccatgttgatga
121  T  P  L  S  P  *   125

451  caataaaatgaatgtgttgcaaaaaaaaaa  480
                      94
```

FIG. 9

BPY2

```
-332                                    aatatctcaggacccaggaccatgtgatatgggcccaacacctggatgatgttactcttctg
-270  cctaggtcatgcgtaaagagggaattagggcatattgcttggcccagtcccgtaatgatatgactctcctgcttgtgccagagccacaga
-180  agtgtgcttggtgacataatctttgaggctgtcacatcaccaagattatattgtatcactggaccagcataaagctgacacttctgacta
 -90  tgcccagccttcaaataatactacactgtataattggctcaacacccaggtgatattgtttccatttacctgagaccagataaaaagccta

   1  ATGATGACGCTTGTCCCCAGAGCCAGGACACGTGCAGGACAGGATCATTACTCTCATCCCTGCCCCAGATTTTCACAGGTGCTGCTTACA
   1  M  M  T  L  V  P  R  A  R  T  R  A  G  Q  D  H  Y  S  H  P  C  P  R  F  S  Q  V  L  L  T

  91  GAGGGCATCATGACATATTGCTTGACAAAGAACCTAAGTGATGTTAATATTCTGCATAGGTTGCTAAAAAATGGGAATGTGAGAAATACC
  31  E  G  I  M  T  Y  C  L  T  K  N  L  S  D  V  N  I  L  H  R  L  L  K  N  G  N  V  R  N  T

 181  TTGCTTCAGTCCAAAGTGGGCTTGCTGACATATTATGTGAAACTGTACCCGGGTGAAGTGACTCTTCTGACTAGGCCCAGCATACAAATG
  61  L  L  Q  S  K  V  G  L  L  T  Y  Y  V  K  L  Y  P  G  E  V  T  L  L  T  R  P  S  I  Q  M

 271  AGATTATGCTGTATCACTGGCTCAGTGTCGAAGCCCAGATCACAGAAGTAAttgtgccatatgtggaacaagcagctaagcaatagataa
  91  R  L  C  C  I  T  G  S  V  S  K  P  R  S  Q  K  *  106

 361  catccatcgtggctctgccttcaaagggaaattttacatatgtcactgggaccatcacccagatgatgtcctgcccactaaaagaattgt
 451  gacataacgctgactgcaaaaactgggtaatgcaactctcctctttattctggagtctgccaaaacaagggattatcacatattgcggag
 541  tccagcacccaggtaaaatttttgtcatatacccagcttcagataccatgcaatgatacaactatcatacctggacccaaagaggagagat
 631  attttgattctcattgccattcttatggccacaagcaaagtaatggttctcatagtggtataaagttcacacagtattatgacactccca
 721  gcgtatcatagaaaatgtgagtagtacaatgagtgttataacagggaacagcaaaccaatgctattgtgattattggattcacacccagc
 811  tgacgcgactatcattctctcacaagaacagaacctgcaaataaagtactaaatctcaccaaaaaaaaaaa   880
```

FIG. 10

XKRY

```
-663                                                                    attaaaaacttctgataaaattacctaagtaca
-630  cacaaacaaaaacatgcccacacaaatcacttaatttctaaaacttttaatttttctgcttctctagtaccttgtattccatcacacagc
-540  aaaatctggcagctccacttccagaatttacttgaactccacagcttatttccgatttcctgttatcaccagagtctaaaacacagttta
-450  tattgcattcacctcctatttttacaccgtaatttcctactttacactctaactttatataaaaaagaaactacctttttcaagatctaatt
-360  cacgcaatttttatttgttcttaattgagacttctttctaggtgctgtcacaccttgtaacgtcagatacaaatgtctctatccaatttca
-270  tgagttccagttatttttatttttaagggaatgtgtatatacatttataaatttgtgtatgtgtgtattcacttattctttatttttatatgt
-180  tttgcatgcatatattcactaaatccctgataatagaaagataacaaatctttttttttctttctttttttgtatgtaaattattttccga
 -90  aggaggtgggttgggagaaatatatcttaacttggcaagtttaaaagagaaagtggccattactaatgaaaattattctctagcattttc

   1  ATGTTTATCTTTAATAGCATTGCTGATGACATATTCCCTCTTATCAGTTGTGTAGGTGCCATTCACTGCAATATACTGGCCATCCGCACT
   1  M  F  I  F  N  S  I  A  D  D  I  F  P  L  I  S  C  V  G  A  I  H  C  N  I  L  A  I  R  T
  91  GGCAACGACTTTGCTGCCATTAAGCTACAGGTGATAAAATTGATCTATCTCATGATATGGCATTCGTTGGTGATTATCTCACCTGTAGTG
  31  G  N  D  F  A  A  I  K  L  Q  V  I  K  L  I  Y  L  M  I  W  H  S  L  V  I  I  S  P  V  V
 181  ACTCTGGCATTCTTCCCTGCATCTCTGAAACAGGGGAGCTTACACTTTCTATTAATCATATATTTTGTATTATTGTTGACACCATGGCTG
  61  T  L  A  F  F  P  A  S  L  K  Q  G  S  L  H  F  L  L  I  I  Y  F  V  L  L  L  T  P  W  L
 271  GAGTTTTCGAAAAGTGGAACTCATCTTCCTAGCAACACAAAAATAATTCCAGCATGGTGGGTAAGTATGGATGCTTATCTTAATCATGCT
  91  E  F  S  K  S  G  T  H  L  P  S  N  T  K  I  I  P  A  W  W  V  S  M  D  A  Y  L  N  H  A
 361  AGTATATGCTGCCATCAATTCTCCTGCTTGTCAGCAGTGAAACTGCAGCTGTCAAATGAGGAATTGATAAGAGACACGAGGTGGGACATA
 121  S  I  C  C  H  Q  F  S  C  L  S  A  V  K  L  Q  L  S  N  E  E  L  I  R  D  T  R  W  D  I
 451  CAATCCTACACTACAGATTTCAGTTTTTAGaaaatgtgataataatattgatatttagtttctttggagggaacgttttaccgaagtgtt
 151  Q  S  Y  T  T  D  F  S  F  *    159
 541  gtgactcaataattgccgtgtagttcatcaaaacctacatattagcctttggctttaagctccgcttctgtcagtatttgcaaccaaggt
 631  ggtcgggcaaagtattgccaggagatactgaaaatcatccagaagcactgtgatattgtgtaagcatctggagaaaattcagttaaaaga
 721  ataaaagtaagcagctgaggaattactatcactcatggagaagggtaggatattttcaataagtgagtatgcaatatccatatatacttt
 811  cacagaacaaagagtaaagaggctgagtgtgactttataaagatactcatgaaaaatataaacaacaaaaccttggaagtagtttctaat
 901  aaaattgatttttctaaaaaaaaaa     925
```

FIG. 11

PTPRY

```
-182
-180  gaagaggagcacaccacaccagaaacagacatcttgcagtgtttcactgtctcaaccttatctgcacagtccgaggtcagtctgagagag                aa
 -90  cttctgagagacccaggatgaagggatgcagtgaggtcaagagcccaaccttctttcactgacacccacctctaaggactcagaagagac

   1  ATGAATAAAATGGGCCTCAACAATCCCAAGAAGAACCACTCAAGGACAATGGGAGCCACTGGGCTTGGCTTCCTACTTCCCTGGAAACAA
   1  M  N  K  M  G  L  N  N  P  K  K  N  H  S  R  T  M  G  A  T  G  L  G  F  L  L  P  W  K  Q
  91  GACAATTTGAATGGCACTGACTGCCAGGGATGCAATATTTTATACTTCTCTGAGACTACGGGGAGCATGTGTTCTGAACTTTCCCTGAAC
  31  D  N  L  N  G  T  D  C  Q  G  C  N  I  L  Y  F  S  E  T  T  G  S  M  C  S  E  L  S  L  N
 181  AGAGGTCTTGAGGCCAGAAGGAAGAAGGATCTTAAAGACTCATTTCTCTGGAGATATGGGAAGGTTGGCTGTATCTCACTTCCACTTCGT
  61  R  G  L  E  A  R  R  K  K  D  L  K  D  S  F  L  W  R  Y  G  K  V  G  C  I  S  L  P  L  R
 271  GAGATGACCGCCTGGATTAACCCACCCCAAATTTCAGAGATTTTCCAAGGCTACCACCAGAGGGTGCACGGAGCTGATGCACTGAGCCTG
  91  E  M  T  A  W  I  N  P  P  Q  I  S  E  I  F  Q  G  Y  H  Q  R  V  H  G  A  D  A  L  S  L
 361  CAAACCAACTCTCTGAGAAGCAGGTTATCTTCACAGTGCCTCGGACAGAGCTTCCTTCTCAGGACACTCGAGAGAGCCGTGGTTTCAGGG
 121  Q  T  N  S  L  R  S  R  L  S  S  Q  C  L  G  Q  S  F  L  L  R  T  L  E  R  A  V  V  S  G
 451  CACTTGGGGACATCTGTGGCCACGTTCATGAAGAAGACTAAGCCTACTTCATCTCAGGACCCGCCCAAGAGTGGCCGCGGCTTTGGGACA
 151  H  L  G  T  S  V  A  T  F  M  K  K  T  K  P  T  S  S  Q  D  P  P  K  S  G  R  G  F  G  T
 541  CCTGCGGTCGGGTCCACCATGAGGATAAAACCTCCTTCTCTTCTGGACATGTCCAGGAGTGGCCGTTGCTACAAGTCACCTGGTGCTACG
 181  P  A  V  G  S  T  M  R  I  K  P  P  S  L  L  D  M  S  R  S  G  R  C  Y  K  S  P  G  A  T
 631  ACCAGGGTGAGAATAAAGACGTCTCCTCAGGACCCTCCCAGGAGAGTACATGGCATTGAGACATCTGGCGGCCAAGTGAGGAAAAGACAC
 211  T  R  V  R  I  K  T  S  P  Q  D  P  P  R  R  V  H  G  I  E  T  S  G  G  Q  V  R  K  R  H
 721  CCTGTCTGCAGCACCCAGAACTGAggaggggcactgccctgggccttacttcccagccctggcctccaattctgaccttacaaaagtgtc
 241  P  V  C  S  T  Q  N  *   247
 811  ccttgagtgaggcagtgaccacgcattgtcacagctaccaaagtgtggtttgcagatgatctgggcttgtttctggcagagattctggta
 901  cagagaaaggagaggcgttgagtggaaccacgatgggctgaggccaggggagacatcacaacctccaacaacacttttttcatgcttta
 991  ataaatcatttttcttagagaactaaagtagttgaaacaatatagaaacatttttaagtaggcataaaaaaaaaa   1066
```

FIG. 12

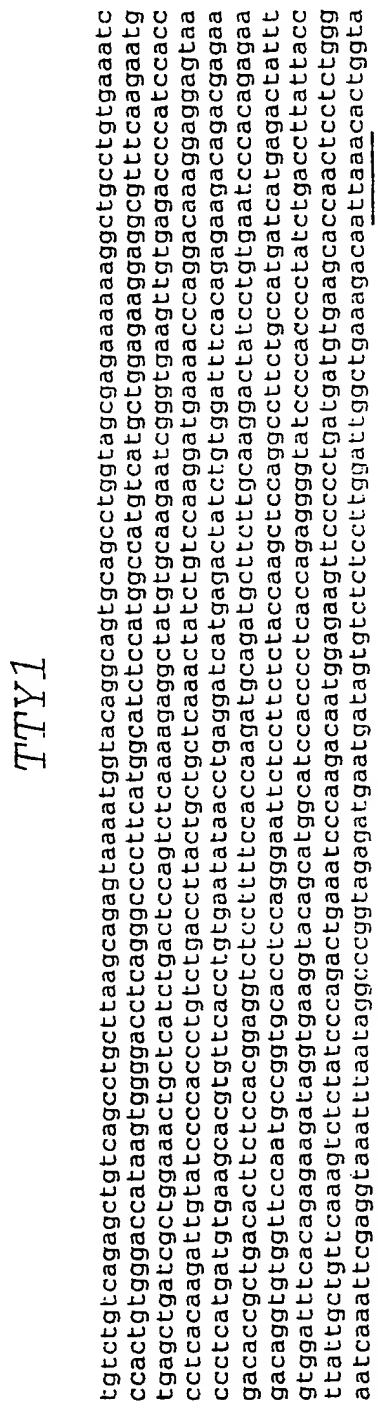

FIG. 13

TTY2

aggcttgccatcaccacagatggcctctgagacactgtttgaaccacatctgcacctgtgagaggccagtttgaggtatgagaacactgt
ttcaatttggacttgcctttgtcttggttcctgcttttcccagatggcacctacccaacccaggatgaatgagtgcagagaggtcaagtg
ccaggccatcttttgctgacacccttttctggtatttcaggtataagtccatcatccaaagactgctcaacatctcaccagaatatattt
caatcctcatggggcatgattctttcacaaaacccctttcaggaatggagtcagaagagtagtttccagagacaacctcacagtcttgga
acggctctgcctcccatgtgatctgaccatggagatggcatataagggccctaagtttgagacttttagggtactgcaatgcgttatcac
aggcagcctttatcctgataccaagccagctctgcctgtaccatttttcctctgcttaggcaggctgacagccctgacaccctggtgctcc
agtttgagtcactatatgtggatgtgctagtcttggggcaatggacctgagctgtgagctgtagctagtgtcacaatgaatgccagcttt
gctagtaacaattcccctttggcttggtagagaagggacctctgtggaggtacaatggtggtgcactgtcacctgtcttctctgtgggat
ccatgggacagttccatgatcctaggagagggtagatgtgagccagcctgaagaaatgtcaagcagagccccaggaatgaagcacaaaat
cactacagatccaaaaggatctgcagaatttgtcaggcctgcctagacattgtaggggttagtcttattgaaatgtgtcccactgtaatt
tccaacttcagccttcctgtgttcccagcagtttctctctcccaggtggggctttctgcagaatgacacagcctcagaagctactgggct
gtgtgttactgtgggagtgttgcgagtgttggatgtcagcatgtgtgtgtggctttgtgtgtgtgtgtaggtgtctgtgtgtgtgtatgt
aaatgaattctgtggatcaggaatcagcaatgactagttaagctgtctgtgaccagccgggttccccatcgtctgcccctgccaaaaaaa
caggtactcttctacaaagaagaggagagcaccacacccaagaacagacatctcccagtgttgcattataaagcagccaacccacagaca
ctagcactctggtctgcatagccccttaatttacctagaattcagttcccagccaagtaggtgcttcatgtcctgagggtgcaatcctc
catcatcttgagatttcatgctggtacagagagtgtgacagcaataaggtcagataggggtgagtatacaacctggtgaagggtggatgg
ggtcccgtaccttcaccagcaaaaagggtgaaaatagatgacacagaatgtgcttccaactccatccccacattcccataattgcaaaat
cagtcaacaacatggcctggtgtttaggtgggagtactccaacctgcaggaagaatttggagtgcaaattgtggccaatctggaaaactc
ctggtttgagggttttaatacctgtagtcaaatggaagtggaatagattgatgctgggtgggttgtggcctccacatttgtgtcctcttt
tactgacttccattgtcctcattggtgtagggcttcctggatctggctcaacatcttccacactaaactcttccctgttcacagaagacc
atcataaaaatgcattgtagaggccctgcaaggaccaggatgaagggagacagtgaggtcaagagcccagccatctttcactgacaccca
cttctgggttctcaggctggctgacaggtctgacagcccatcacgaaagcctgcatactcttagacacaaggactgagctatgggctcca
gctagcatcacaatgaaggccaccattgcctagggataagtccctgtgactttgtggataagaactccgtggagccaatccaaggagaga
cactatcattcatctctaccgggcctattaaatttacctcgaatttgattcccagaggagttggtgcttcacatcatcagggggaacttc
tccattgtcttgggatttcagtctgggatagagactttgaacagcaataagtttccttggtctggctcaacgtcttctaaactcaacatt
ccccagttcatggaaaatgatcctcatgggattctattgtaaaagctgcatgattcctggagaatgttttattgtcctggagtcaaccca
aaaatcaacactaccagtcatgttgcagagctccttgaattaaccttgaattcagtttccagctgagcagctgcttcacgttgtgagggg
gcaatcctccatcatcctgggatttcattctgggacacagagtttgagcagcaataaggctgggtccacattgcccctcaacagcattag
tggacatgattgtcagacttgcaatttccgcagacaccttctgtgaacatttttcaacatcatctacatgagtgagagacccgttcgaca
tgtaagaatactgcttgactttggacctgcctttgtcgtggttcctgccttttctcatagatcccctgccaggcccaggatgataggaggc
aatgaagtcaagggccgagccccattcattgaaagctgactctggggtctcgggtataattccatcacataaaatcccctcaacaactca
ccagactatattccaatctccatggaacctgattcttgcacacagcctctttcaggaatggagtcagaagagcagtcttcagagaccacc
tcagtttggaaaagcctcctccttcagtggttcccagccatggagtcatcgtgaaggggctccatggtcaataattttacggtactgcac
ttggttatcacagacagacttttcatgatagcatgccatctctgtctatatcattttcctctgcttaggcaggctgacaactctgacag
ccaggggcccgaatctacctggcaaatgtgcatgctctactctcagtgcaaaaggcctgtttgggagttctgactagtgtcacaataaat
gccgccattgcctagtgaaaaaaaaaa

FIG. 14

Human *CDYL (CDY Like)*

ggagagaggacctatttctacctaaggacattcccggaaggcaatgggtttcaaacaatatcct
gaagagactcatctcggggaactaagcaggtggtaatcagagaacacagagcccccggaagaat
tttATGGCATTTCAGGCAAGCCACAGGCCAGCCTGGGGAAAAAGCAGGAAGAAAAACTGGCAAT
ACGAGGGCCCAACCCAAAAGTTATTCCTGAAGAGAAACAACGTGTCAGCACCAGATGGGCCTTC
AGACCCCAGCATCTCCGCGAGCAGTGAGCAAAGCGGGGCACAGCAGCCTCCCGGTTTACAGGTT
GAAAGGATTGTTGACAAAAGGAAAAATAAAAAAGGGAAGACAGAGTATTTGGTTCGGTGGAAAG
GCTATGACAGCGAGGACGACACTTGGGAGCCGGAACAGCACCTCGTGAACTGTGAGGAATACAT
CCACGACTTCAACAGACGCCACACGGAGAAGCAGAAGGAGAGCACATTGACCAGAACAAACAGG
ACCTCTCCCAACAATGCTAGGAAACAAATCTCCAGATCCACCAACAGCAACTTTTCTAAGACCT
CTCCTAAGGCACTCGTGATTGGGAAAGACCACGAATCCAAAAACAGCCAGCTGTTTGCTGCCAG
CCAGAAGTTCAGGAAGAACACAGCTCCATCTCTCTCCAGCCGGAAGAACATGGACCTAGCGAAG
TCAGGTATCAAGATCCTCGTGCCTAAAAGCCCCGTTAAGAGCAGGACCGCAGTGGACGGCTTTC
AGAGCGAGAGCCCTGAGAAACTGGACCCCGTCGAGCAGGGTCAGGAGGACACAGTGGCACCCGA
AGTGGCAGCGGAAAAGCCGGTCGGAGCTTTATTGGGCCCCGGTGCCGAGAGGGCCAGGATGGGG
AGCAGGCCCAGGATACACCCACTAGTGCCTCAGGTGCCCGGCCCTGTGACTGCAGCCATGGCCA

FIG. 15A

CAGGCTTAGCTGTTAACGGGAAAGGTACATCTCCGTTCATGGATGCATTAACAGCCAATGGGAC
AACCAACATACAGACATCTGTTACAGGAGTGACTGCCAGCAAAAGGAAATTTATTGACGACAGA
AGAGACCAGCCTTTTGACAAGCGATTGCGTTTCAGCGTGAGGCAAACAGAAAGTGCCTACAGAT
ACAGAGATATTGTGGTCAGGAAGCAGGATGGCTTCACCCACATCTTGTTATCCACAAAGTCCTC
AGAGAATAACTCACTAAATCCAGAGGTAATGAGAGAAGTCCAGAGTGCTCTGAGCACGGCCGCT
GCCGATGACAGCAAGCTGGTACTGCTCAGCGCCGTTGGCAGCGTCTTCTGTTGTGGACTTGACT
TTATTTATTTTATACGACGTCTGACAGATGACAGGAAAAGAGAAAGCACTAAAATGGCAGAAGC
TATCAGAAACTTCGTGAATACTTTCATTCAATTTAAGAAGCCCATTATTGTAGCAGTCAATGGC
CCAGCCATTGGTCTAGGAGCATCTATATTGCCTCTTTGCGATGTGGTTTGGGCTAATGAAAAGG
CTTGGTTTCAAACACCCTATACCACCTTCGGACAGAGTCCAGATGGCTGTTCTACCGTTATGTT
TCCCAAGATAATGGGAGGAGCATCTGCAAACGAGATGCTGCTCAGTGGACGGAAGCTGACAGCG
CAGGAGGCGTGTGGCAAGGGCCTGGTCTCCCAGGTGTTTTGGCCCGGGACGTTCACTCAGGAAG
TGATGGTTCGCATTAAGGAGCTTGCCTCGTGCAATCCAGTTGTGCTTGAGGAATCCAAAGCCCT
CGTGCGCTGCAACATGAAGATGGAGCTGGAGCAGGCCAACGAGAGGGAGTGTGAGGTGCTGAAG
AAAATCTGGGGCTCGGCCCAGGGGATGGACTCCATGTTAAAGTACTTGCAGAGGAAGATCGATG

FIG. 15B

AGTTCTGAgtgtcgggctgcccactggtgacaccgggatcgggctgagcaggagaacatcaccg
gctccagttcccctgatccattctcacagcctgaaacaagctcacccgtagcttacgcttggaa
gcaggactgggaacatccacgctatttattatcgaggagttttaaagtactgtaactttaaaat
aaataactacaaagcttctttgtcvaaacgtcattattttatacttatatacacgcaggtgtaa
aagtataaaggtgagcactagactgctcttagaagctctaatttttgttttctttggctagtac
tgtataaaaaacagaattgtgttttattggttttggatgacagaaaagtctggaataatgtttg
ttttcctcatttcttccttctagaacacagaatctaaggggtgttagccagcctcgcctccct
gccccacgtagagacacagagtgatgtgaggcgttggctttttctccaagaaggtacagatacc
tcagattcgggaaactcaaaatcaaaagacttagcttctaggataaatacttctgatgaaaaat
ccgctgaggagcataccccaaaccagacatatgcttaggattcatgctgagatatcaattggtt
tcccctttcttttaaaatacgtccagttcttacccagttaacatgaagaaaccactgtctctag
aagaaagcttgttttgcagtattagtgaatcactgaatagcttaagtatgactatctaagttat
aagttagtctttagtgggttttaaatagtttttctgacccttctgaaaaataactacataagtg
cttcttgttgctgggtgagaaatactactttatagacagttttggttttctgtttgcagatatg
attgatgtatttcaccaaaataaaatatttttatgtttataaagtgtaatttttaggttcactt
agaatatattttatttaataagttaaaattcttttggcacactattaaatgcaaaaactccttt
c

FIG. 15C

Mouse *Cdyl (CDY like)*

ctttgaggtggtttagcatcccacttgttccttgaggacatctgttcctacctaagagcactcacc
tgagatgctcaaaggtccagaagaaacacttctcgggtgacaaagcaggtggtgaccagagaacag
aggccccccaaaaattttatggcattcaaggcaaagcacagccaacccggagggaaagcaagagtc
cagcctggaaatacatagcccaacccgaaggttatctctgaaggaaaacaATGGGCATAGGCAATA
GCCAGCCTAATTCACAGGAAGCCCAGCTCTGCACACTTCCAGAGAAAGCTGAACAACCTACTGATG
ATAACACCTGCCAGCAAAATAATGTGGTTCCTGCAACAGTCTCAGAACCCGATCAAGCGTCCCCTG
CAATTCAAGACGCGGAGACTCAGGTGGAAAGTATCGTTGACAAAAGGAAAAACAAGAAAGGGAAGA
CAGAATATCTGGTGCGGTGGAAAGGCTATGACAGTGAGGATGACACGTGGGAGCCTGAGCAGCACC
TGGTGAACTGTGAGGAATACATCCATGACTTCAACCGGCGCCACAACGAGAGGCAAAAGGAAGGTA
GCCTGGCTCGTGCCAGCAGAGCCTCCCCCAGCAACGCCCGGAAGCAGATTTCCAGGTCCACCCACA
GCACTCTCTCCAAGACCAACTCCAAAGCACTTGTGGTAGGCAAAGATCATGAGTCCAAAAGCAGCC
AGCTGTTGGCTGCCAGCCAGAAGTTCAGGAAAAACCCAGCCCCATCTCTTGCAAACCGCAAGAACA
TGGACCTCGCCAAGTCAGGGATCAAAATTCTCGTGCCTAAGAGCCCCGTTAAGGGCAGGACCTCGG
TTGATGGCTTTCAGGGGGAGAGCCCCGAGAAGCTGGACCCTGTGGATCAGGGTGCCGAGGACACTG
TAGCCCCAGAGGTGACTGCAGAGAAGCCCACTGGGGCTTTGCTGGGCCCTGGTGCGGAGCGAGCCA

FIG. 16A

GGATGGGGAGCAGGCCCCGAATACATCCACTAGTGCCTCAGGTTTCTGGCCCCGTGACTGCTGCCA
TGGCCACAGGCTTAGCTGTTAATGGAAAAGGTACATCTCCATTCATGGATGCGCTAGCAGCCAACG
GAACAGTCACCATACAGACATCCGTAACAGGAGTGACAGCCGGGAAAAGGAAATTTATTGACGACA
GAAGAGACCAACCTTTTGACAAGCGGTTGCGTTTCAGTGTGAGGCAGACAGAGAGTGCCTACAGAT
ACAGAGATATTGTCGTCAGGAAGCAAGATGGCTTCACCCACATCTTGTTATCCACAAAATCGTCAG
AGAATAACTCACTAAACCCAGAGGTGATGAAAGAAGTRCAGAGCGCCCTGAGCACAGCTGCAGCCG
ACGACAGCAAGCTGGTTCTGCTCAGCGCCGTGGGCAGCGTCTTCTGCTGTGGTCTGGACTTTATTT
ATTTTATTCGGCGCCTCACAGATGACCGAAAGAGAGAAAGCACTAAAATGGCAGACGCTATCAGAA
ACTTCGTGAATACTTTCATTCAGTTTAAGAAGCCTATTATTGTAGCTGTTAATGGCCCAGCCATTG
GACTAGGAGCATCCATATTGCCTCTTTGTGATGTGGTTTGGGCTAACGAAAAGGCTTGGTTTCAAA
CACCCTATACCACCTTCGGACAGAGTCCAGATGGCTGCTCTACCGTTATGTTTCCCAAGATTATGG
GAGGAGCATCTGCGAATGAAATGCTGTTCAGTGGGCGGAAGTTGACGGCACAGGAGGCCTGTGGCA
AGGGTCTGGTCTCCCAGGTGTTTTGGCCAGGAACCTTCACACAGGAAGTCATGGTTCGAATCAAGG
AGCTGGCTTCATGTAACCCAGTTGTCCTGGAGGAATCCAAAGCCCTGGTGCGCTGCAATATGAAGA

FIG. 16B

TGGAGCTAGAGCAGGCCAATGAGAGAGAATGTGAAGTGCTGAAGAAGATCTGGGGCTCCGCCCAGG
GCATGGACTCCATGTTAAAGTACTTACAGAGGAAAATCGATGAGTTCTGAtgggcaggctgagcag
gacatcggtggctcccacttgctacgtcgtcctgcagtggctcgtgcttggaggcagaactggaaa
catccgagctatttattgccgcggagtttttaagtactgtaactttaaaataaatacaaagcttct
ttgtctaagcgtctttatttatactcatgtatacacaagtataaaaatgtaattgagcactaggc
tgctcttggaagctctaattttcttgtaagctagttgtggatttttgttttgttttttgttttttaaa
aggaattatgttttcattttgggtgacagaagagtttgaaataatgtttgttttactctttttttt
tttccttaaatctagatcacagaccctcaaaattactagccagccttctccccctccctctactga
aacatgtagaaatacttaaacatgttcctgcctctaggggggaggggggaggtgtgagtacctcaat
gctgaaaacagttctgatcaaacttaagaccaacctggtaaaaaaagcatcactgatggaaaatcc
cacccacgggggcgtgggtttctgctgaaatgcccgccgctctacctttcttactgtcccattctt
acccagccaccgtgaagagcccagtgtctggaggaaagcaggtggtccagtgtctgtgagtcactc
cgtagctcgagtgttacttgctaagttatgaattagcattagtgggtttaaatagtttttctgacc
ctttttgaaaaataactacataagtactccttgtggctgggtgagaaatactactttgcatagttt
tgtttgtctatctgcagatatgattgctgtattacaccaaaagtatttttttatgtttataaagtgt
aatttttaggttcacttagaatatattttatttaatttaaaattctcttggcacactattaaatac
gtaaactcctttc

FIG. 16C

VCP2r (VCP with 2 repeats)

gttgcgagacgttgagctgcggaagATGAGTCCAAAGCCGAGAGCCTCGGGACCTCCGGCCAAGGCCAC
GGAGGCAGGAAAGAGGAAGTCCTCCTCTCAGCCGAGCCCCAGTGACCCGAAGAAGAAGACTACCAAGGT
GGCCGAGAAGGGAAAAGCAGTTCGTAGAGGGAGACGCGGGAAGAAAGGGGCTGCGACAAAGATGGCGGC
CGTGACGGCACCTGAGGCGGAGAGCGGGCCAGCGGCACCCGGCCCCAGCGACCAGCCCAGCCAGGAGCT
CCCTCAGCACGAGCTGCCGCCGGAGGAGCCAGTGAGCGAGGGGACCCAGCACGACCCCCCGAGTCAGGA
GGCCGAGCTGGAGGAACCACTGAGTCAGGAGAGCGAGGTGGAAGAACCACTGACTGTGTGGATGGCCAG
CTTTTCCCCTGTCTCCGAGAGCAGCGACTAAgttcaggcccagccgccagacctcagagatctcaccag
cggggtgcttgccattctgaagataataaaatgaatgtgttgcaaattgaaaaaaaaa

FIG. 17A

VCP8r (VCP with 8 repeats)

cggaagATGAGTCCAAAGCCGAGAGCCTCGGGACCTCCGGCCAAGGCCACGGAGGCAGGAAAGAGGAAG
TCCTCCTCTCAGCCGAGCCCCAGTGACCCGAAGAAGAAGACTACCAAGGTGGCCAAGAAGGGAAAAGCA
GTTCGTAGAGGGAGACGCGGGAAGAAAGGGGCTGCGACAAAGATGGCGGCCGTGACGGCACCTGAGGCG
GAGAGCGGGCCAGCGGCACCCGGCCCCAGCGACCAGCCCAGCCAGGAGCTCCCTCAGCACGAGCTGCCG
CCGGAGGAGCCAGTGAGCGAGGGGACCCAGCACGACCCCCTGAGTCAGGAGGCCGAGCTGGAGGAACCA
CTGAGTCAGGAGAGCGAGGTGGAAGAACCACTGAGTCAGGAGAGCCAGGTGGAGGAACCACTGAGTCAG
GAGAGCGAGGTGGAGGAACCGCTGAGTCAGGAGAGCCAGGTGGAAGAACCACTGAGTCAGGAGAGCGAG
GTGGAGGAACCACTGAGTCAGGAGAGCCAGGTGGAGGAACCACTGAGTCAGGAGAGCGAGATGGAAGAA
CTACCGAGTGTGTAGACGGCCAGCTACTCCCCTATCTCCGAGAGCAGCGACTAAgttcaggcccagccg
ccagacctcagagatctcaccagcggggtgcttgccattctgaagataataaaatgaatgtgttgcaaa
ttgaaaaaaaaaa

FIG. 17B

VCP10r (VCP with 10 repeats)

cgttgcgagacgttgagctgcggaagATGAGTCCAAAGCCGAGAGCCTCGGGACCTCCGGCCAAGGCCA
CGGAGGCAGGAAAGAGGAAGTCCTCCTCTCAGCCGAGCCCCAGTGACCCGAAGAAGAAGACTACCAAGG
TGGCCAAGAAGGGAAAAGCAGTTCGTAGAGGGAGACGCGGGAAGAAAGGGGCTGCGACAAAGATGGCGG
CCGTGACGGCACCTGAGGCGGAGAGCGGGCCAGCGGCACCCGGCCCCAGCGACCAGCCCAGCCAGGAGC
TCCCTCAGCACGAGCTGCCGCCGGAGGAGCCAGTGAGCGAGGGGACCCAGCACGACCCCCTGAGTCAGG
AGGCCGAGCTGGAGGAACCACTGAGTCAGGAGAGCGAGGTGGAAGAACCACTGAGTCAGGAGAGCCAGG
TGGAGGAACCACTGAGTCAGGAGAGCGAGGTGGAAGAACCACTGAGTCAGGAGAGCCAGGTGGAGGAAC
CACTGAGTCAGGAGAGCGAGGTGGAGGAACCACTGAGTCAGGAGAGCCAGGTGGAGGAACCACTGAGTC
AGGAGAGCGAGATGGAAGAACCACTGAGTCAGGAGAGCCAGGTGGAGGAACCACCGAGTCAGGAGAGCG
AGATGGAAGAACTACCGAGTGTGTAGACGGCCAAGTACTCCCCTATCTCCGAGAGCAGCGACTAAgttc
aggcccagccgccagacctcagagatctcaccagcggggtgcttgccattctgaagataataaaatgaa
tgtgttgcaaattgaaaaaaaaaa

FIG. 17C

INTERNATIONAL SEARCH REPORT

Inter[illegible]onal Application No
P[illegible]US 98/07115

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 00375 A (IMP CANCER RES TECH) 9 January 1992 see the whole document ---	1
X	ZHANG J. ET AL.: "Molecular isolation and characterization of an expressed gene from the human Y chromosome" HUMAN MOLECULAR GENETICS, vol. 1, no. 9, December 1992, pages 717-726, XP002080218 see the whole document --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 October 1998	17. 12. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna[illegible]nal Application No
PCT/US 98/07115

C.(Continuation) DOCUMENTS CONSI[illegible]ED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MA K. ET AL.: "A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis" CELL, vol. 75, no. 7, 31 December 1993, pages 1287-1295, XP002017338 cited in the application see the whole document	1,2
X	WO 95 11300 A (MEDICAL RES COUNCIL ;CHANDLEY ANN CHESTER (GB); KUN MA (GB); SHARK) 27 April 1995 see the whole document	1,2
A	WO 97 10267 A (PROMEGA CORP ;KENT MARIJO G (US); AGULNIK ALEXANDER I (US)) 20 March 1997 see the whole document	1-4,8
A	PAGE D. ET AL.: "The sex-determining region of the human Y chromosome encodes a finger protein" CELL, vol. 51, no. 6, 24 December 1987, pages 1091-1104, XP002080219 cited in the application see the whole document	1-4,8
A	WO 96 41007 A (PROMEGA CORP) 19 December 1996 see the whole document	1-4,8
A	FOOTE S. ET AL.: "The human Y chromosome: overlapping DNA clones spanning the euchromatic region" SCIENCE, vol. 258, 2 October 1992, pages 60-66, XP002080220 see the whole document	1-4,8
P,X	LAHN B. AND PAGE D.: "Functional coherence of the human Y chromosome" SCIENCE, vol. 278, 24 October 1997, pages 675-680, XP002080221 see the whole document	1-4,8

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/07115

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4,8 partially (subject 1. on continutation-sheet)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-4,8 partially

Isolated DNA which occurs on the non-recombining region of the human Y chromosome or the complement thereof, being testis-specific and optionally occuring in multiple copies on the Y chromosome.
Said DNA being the CDY gene, a characteristic portion, a probe or complement thereof, a DNA which hybridizes thereto under stringent conditions.
Said DNA having the SEQ ID NO:37,38 and coding for the amino acid of SEQ ID NO:39,40.

2. Claims: 1-4,8 partially

idem for BPY 1, SEQ ID NO:41,42

3. Claims: 1-4,8 partially

idem for BPY 2, SEQ ID NO:43,44

4. Claims: 1-4,8 partially

idem for XKRY, SEQ ID NO:45,46

5. Claims: 1-4,8 partially

idem for PTPRY, SEQ ID NO:47,48

6. Claims: 1-4,8 partially

idem for TTY 1, SEQ ID NO:49

7. Claims: 1-4,8 partially

idem for TTY 2, SEQ ID NO:50

8. Claims: 5-7,9 partially

Isolated DNA which occurs on the non-recombining region of the human Y chromosome or the complement thereof, not being testis-specific and having a homolog on the human X chromosome.
Said DNA being the DBY gene; a characteristic portion, a probe or complement thereof, a DNA which hybridizes thereto under stringent conditions.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Said DNA having the SEQ ID NO:17 and coding for the amino acid of SEQ ID NO:18.

9. Claims: 5-7,9 partially

idem for TPRY, SEQ ID NO:19,20,21,22,23,24

10. Claims: 5-7,9 partially

idem for TB4Y, SEQ ID NO:26,28

11. Claims: 5-7,9 partially

idem for EIF1AY, SEQ ID NO:30,32

12. Claims: 5-7,9 partially

idem for DFFRY, SEQ ID NO:34,36

INTERNATIONAL SEARCH REPORT

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PCT/US 98/07115

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